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<b>(54) Title:</b> BIOSYNTHESIS OF MEDIUM CHAIN LENGTH POLYHYDROXYALKANOATES		
<b>(57) Abstract</b>  Nucleic acids, proteins, and methods for the biosynthesis of polyhydroxyalkanoate polymer materials are disclosed. In a preferred embodiment, expression of a polyhydroxyalkanoate synthase protein with a peroxisome targeting peptide results in the biosynthesis of medium chain length polyhydroxyalkanoates. In an alternative embodiment, exogenous addition of fatty acids to a plant or cell containing a peroxisome targeted polyhydroxyalkanoate synthase protein leads to the biosynthesis of novel polymeric materials.		

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## BIOSYNTHESIS OF MEDIUM CHAIN LENGTH POLYHYDROXYALKANOATES

### FIELD OF THE INVENTION

5       The invention relates to the biosynthesis of polymers and more specifically to the biosynthesis of polyhydroxyalkanoate polymers in plants. In particular, a transgenic plant producing peroxisome- or glyoxysome-targeted polyhydroxyalkanoate synthase resulting in the production of polyhydroxyalkanoate materials.

### BACKGROUND OF THE INVENTION

10       PHAs are bacterial polyesters that accumulate in a wide variety of bacteria. These polymers have properties ranging from stiff and brittle plastics to rubber-like materials, and are biodegradable. Because of these properties, PHAs are an attractive source of nonpolluting plastics and elastomers.

15       Currently, there are approximately a dozen biodegradable plastics in commercial use that possess properties suitable for producing a number of specialty and commodity products (Lindsay, *Modern Plastics* 2: 62 (1992)). One such biodegradable plastic in the polyhydroxyalkanoate (PHA) family that is commercially important is Biopol™, a random copolymer of 3-hydroxybutyrate (3HB) and 3-hydroxyvalerate (3HV). This bioplastic is used to produce biodegradable molded material (e.g., bottles), films, coatings, and in drug  
20       release applications. Biopol™ is produced via a fermentation process employing the bacterium *Alcaligenes eutrophus* (Byrom, *Trends Biotechnol.* 5: 246 (1987)). The current market price is \$6-7/lb, and the annual production is 1,000 tons. By best estimates, this price can be reduced only about 2-fold via fermentation (Poirier et al., *Bio/Technology* 13: 142 (1995)). Competitive synthetic plastics such as polypropylene and polyethylene cost  
25       about 35-45¢/lb (Layman, *Chem. & Eng. News*, p. 10 (Oct. 31, 1994)). The annual global demand for polyethylene alone is about 37 million metric tons (Layman, *Chem. & Eng. News*, p. 10 (Oct. 31, 1994)). It is therefore likely that the cost of producing P(3HB-co-3HV) by microbial fermentation will restrict its use to low-volume specialty applications.

Polyhydroxyalkanoate (PHA) is a family of polymers composed primarily of R-3-hydroxyalkanoic acids (Anderson, A. J. & Dawes, E. A. *Microbiol. Rev.* 54: 450-472. (1990); Steinbüchel, A. in *Novel Biomaterials from Biological Sources*, ed. Byrom, D. (MacMillan, New York), pp. 123-213. (1991); Poirier, Y. Nawrath, C. & Somerville, C. *Bio/Technology* 13: 143-150 (1995)). Polyhydroxybutyrate (PHB) is the most well characterized PHA. High molecular weight PHB is found as intracellular inclusions in a wide variety of bacteria (Steinbüchel, A. in *Novel Biomaterials from Biological Sources*, ed. Byrom, D. (MacMillan, New York), pp. 123-213. (1991)). In *Alcaligenes eutrophus*, PHB typically accumulates to 80% dry weight with inclusions being typically 0.2-1 µm in diameter. Small quantity of PHB oligomers of approximately 150 monomer units are also found associated with membranes of bacteria and eukaryotes, where they form channels permeable to calcium (Reusch, R. N., *Can. J. Microbiol.* 41 (Suppl. 1): 50-54 (1995)). High molecular weight PHAs have the properties of thermoplastics and elastomers. Numerous bacteria and fungi can hydrolyze PHAs to monomers and oligomers, which are metabolized as a carbon source. PHAs have, thus, attracted attention as a potential source of renewable and biodegradable plastics and elastomers. PHB is a highly crystalline polymer with rather poor physical properties, being relatively stiff and brittle (de Koning, G., *Can. J. Microbiol.* 41 (Suppl. 1): 303-309 (1995)). In contrast, PHA copolymers containing monomer units ranging from 3 to 5 carbons for short-chain-length PHA (SCL-PHA), or 6 to 14 carbons for medium-chain-length PHA (MCL-PHA), are less crystalline and more flexible polymers (de Koning, G., *Can. J. Microbiol.* 41 (Suppl. 1): 303-309 (1995)).

PHB has been produced in the plant *Arabidopsis thaliana* expressing the *A. eutrophus* PHB biosynthetic enzymes (Poirier, Y., et al., *Science* 256: 520-523 (1992); Nawrath, C., et al., *Proc. Natl. Acad. Sci. U.S.A.* 91: 12760-12764 (1994)). In plants expressing the PHB pathway in the plastids, leaves accumulated up to 14% PHB per gram dry weight (Nawrath, C., et al., *Proc. Natl. Acad. Sci. U.S.A.* 91: 12760-12764 (1994)). High-level synthesis of PHB in plants opened the possibility of utilizing agricultural crops as a suitable system for the production of PHAs on a large scale and at low cost (Poirier, Y. et al., *Bio/Technology* 13: 143-150 (1995); Poirier, Y., et al., *FEMS Microbiol. Rev.* 103: 237-246 (1992); Nawrath, C., et al. *Molecular Breeding* 1: 105-22 (1995)). PHB was also

shown to be synthesized in insect cells expressing a mutant fatty acid synthase (Williams, M. D., et al., *Appl. Environ. Microbiol.* 62: 2540-2546 (1996)), and in yeast expressing the *A. eutrophus* PHB synthase (Leaf, T. A., et al. *Microbiol.* 142: 1169-1180 (1996)).

A number of pseudomonads, including *Pseudomonas putida* and *Pseudomonas*  
5 *aeruginosa*, accumulate MCL-PHAs when cells are grown on alkanolic acids (Anderson, A. J. & Dawes, E. A. *Microbiol. Rev.* 54: 450-472. (1990); Steinbüchel, A. in *Novel Biomaterials from Biological Sources*, ed. Byrom, D. (MacMillan, New York), pp. 123-213. (1991); Poirier, Y. Nawrath, C. & Somerville, C. *Bio/Technology* 13: 143-150 (1995)). The nature of the PHA produced is related to the substrate used for growth and is typically  
10 composed of monomers which are 2n carbons shorter than the substrate. These studies indicate that MCL-PHAs are synthesized by the PHA synthase from 3-hydroxyacyl-CoA intermediates generated by the  $\beta$ -oxidation of alkanolic acids (Huijberts, G. N. M., et al. *Appl. Environ. Microbiol.* 58: 536-544 (1992); Huijberts, G. N. M., et al., *J. Bacteriol.* 176: 1661-1666 (1994)).

15 There exists a need for novel methods towards the biosynthesis of polyhydroxyalkanoate materials suitable for commercial applications. Towards this goal, this patent application discloses the materials and methods for the use of a peroxisome targeted polyhydroxyalkanoate synthase protein in the biosynthesis of polyhydroxyalkanoate polymers. Localization in the peroxisomes allow for the utilization  
20 of intermediates from the lipid  $\beta$ -oxidation pathway. Plants expressing a *P. aeruginosa* polyhydroxyalkanoate synthase modified for peroxisome targeting produce PHA containing saturated and unsaturated 3-hydroxyalkanoic acids ranging from 6 to 16 carbons. Polyhydroxyalkanoate granules are found within the glyoxysomes or leaf-type peroxisomes of dark-and light-grown plants, respectively, as well as in the vacuoles.

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#### SUMMARY OF THE INVENTION

The invention is directed towards materials and methods for the biosynthesis of polyhydroxyalkanoate polymers. More particularly, a fusion protein comprising a

polyhydroxyalkanoate synthase protein subunit and a peroxisome targeting protein subunit renders a host cell or plant capable of producing polyhydroxyalkanoate polymer materials.

In one embodiment, the invention provides a non-naturally occurring fusion protein comprising a peroxisome targeting protein subunit and a polyhydroxyalkanoate synthase protein subunit. Generally, the peroxisome targeting protein subunit and the polyhydroxyalkanoate synthase protein subunit may be any subunit suitable for participation in the invention. The peroxisome targeting subunit may be an N-terminal or C-terminal subunit. The N-terminal subunit is preferably PTS2. The C-terminal peroxisome targeting subunit preferably comprises a tripeptide. The first amino acid in the N-terminus to C-terminus direction is preferably S, A, or P. The second amino acid in the N-terminus to C-terminus direction is preferably K, R, S, or H. The third amino acid in the N-terminus to C-terminus direction is L, M, I, or F. More preferably, the C-terminal peroxisome targeting subunit comprises ARM, SRM, SKL, ARL, SRL, PSI, or PRM. The peroxisome targeting subunit is preferably at least 70% identical to SEQ ID NO:14, more preferably at least 80% identical to SEQ ID NO:14, even more preferably at least 90% identical to SEQ ID NO:14, and most preferably is SEQ ID NO:14. The polyhydroxyalkanoate synthase protein subunit is preferably a *Pseudomonas* subunit, and more preferably a *Pseudomonas aeruginosa* subunit. The polyhydroxyalkanoate synthase protein subunit may preferably be either a PHAC1 or PHAC2 subunit. The PHAC1 subunit is preferably at least 70% identical to SEQ ID NO:2, more preferably at least 80% identical to SEQ ID NO:2, even more preferably at least 90% identical to SEQ ID NO:2, and most preferably is SEQ ID NO:2. The PHAC2 subunit is preferably at least 70% identical to SEQ ID NO:4, more preferably at least 80% identical to SEQ ID NO:4, even more preferably at least 90% identical to SEQ ID NO:4, and most preferably is SEQ ID NO:4. The fusion protein is preferably at least 70% identical to SEQ ID NO:18 or SEQ ID NO:20, more preferably at least 80% identical to SEQ ID NO:18 or SEQ ID NO:20, even more preferably at least 90% identical to SEQ ID NO:18 or SEQ ID NO:20, and most preferably is SEQ ID NO:18 or SEQ ID NO:20.

In an alternative embodiment, the invention encompasses a nucleic acid segment encoding a non-naturally occurring fusion protein. The nucleic acid segment preferably comprises a nucleic acid sequence encoding a peroxisome targeting protein subunit, and

nucleic acid sequence encoding a polyhydroxyalkanoate synthase protein subunit. The nucleic acid sequence encoding a peroxisome targeting protein subunit preferably comprises at least a 6 contiguous nucleic acid sequence from SEQ ID NO:13. The length of the contiguous nucleic acid sequence may be 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, etcetera, 50, 51, 52, etcetera, 100, 101, 102, etcetera, up to and including the entire length of SEQ ID NO:13. The nucleic acid sequence encoding a peroxisome targeting protein subunit is preferably at least 70% identical to SEQ ID NO:13, more preferably at least 80% identical to SEQ ID NO:13, even more preferably at least 90% identical to SEQ ID NO:13, and most preferably is SEQ ID NO:13. The nucleic acid sequence encoding a peroxisome targeting protein subunit preferably hybridizes to SEQ ID NO:13. The nucleic acid sequence encoding a polyhydroxyalkanoate synthase protein subunit preferably comprises at least a 6 contiguous nucleic acid sequence from SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:15, or SEQ ID NO:16. The length of the contiguous nucleic acid sequence may be 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, etcetera, 50, 51, 52, etcetera, 100, 101, 102, etcetera, up to and including the entire length of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:15, or SEQ ID NO:16. The nucleic acid sequence encoding a polyhydroxyalkanoate synthase protein subunit is preferably at least 70% identical to SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:15, or SEQ ID NO:16, more preferably at least 80% identical to SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:15, or SEQ ID NO:16, even more preferably at least 90% identical to SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:15, or SEQ ID NO:16, further preferably is SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:15, or SEQ ID NO:16, and most preferably is SEQ ID NO:15 or SEQ ID NO:16. The nucleic acid sequence encoding a polyhydroxyalkanoate synthase protein subunit preferably hybridizes to SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:15, or SEQ ID NO:16. The encoded peroxisome targeting protein subunit may be an N-terminal or C-terminal peroxisome targeting protein subunit. The encoded N-terminal peroxisome targeting subunit is preferably PTS-2. The encoded C-terminal peroxisome targeting protein subunit preferably comprises a tripeptide. The tripeptide preferably comprises a first amino acid in the N-terminus to C-terminus direction being S, A, or P; a second amino acid in the N-terminus to C-terminus direction being K, R, S, or H; and a third amino acid in the N-terminus to C-terminus direction being L, M, I, or F. The encoded tripeptide preferably is ARM, SRM, SKL, ARL, SRL, PSI, or PRM. The nucleic acid

sequence encoding a polyhydroxyalkanoate synthase protein subunit preferably encodes at least a 5 contiguous amino acid sequence from SEQ ID NO:2 or SEQ ID NO:4. The length of the contiguous nucleic acid sequence may be 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, etcetera, 50, 51, 52, etcetera, 100, 101, 102, etcetera, up to and including the entire  
5 length of SEQ ID NO:2 or SEQ ID NO:4. The nucleic acid sequence encoding a polyhydroxyalkanoate synthase protein subunit preferably encodes an amino acid sequence at least 70% identical to SEQ ID NO:2 or SEQ ID NO:4, more preferably at least 80% identical to SEQ ID NO:2 or SEQ ID NO:4, even more preferably at least 90% identical to SEQ ID NO:2 or SEQ ID NO:4, and most preferably is SEQ ID NO:2 or SEQ ID NO:4.

10 In an alternative embodiment, the invention discloses a recombinant vector comprising in the 5' to 3' direction a) a promoter that directs transcription of a structural nucleic acid sequence encoding a non-naturally occurring fusion protein, wherein the fusion protein comprises a peroxisome targeting protein subunit and a polyhydroxyalkanoate synthase protein subunit, b) a structural nucleic acid sequence encoding a non-naturally  
15 occurring fusion protein, wherein the fusion protein comprises a peroxisome targeting protein subunit and a polyhydroxyalkanoate synthase protein subunit, and c) a 3' transcription terminator. The recombinant vector may further comprise a 3' polyadenylation signal sequence that directs the addition of polyadenylate nucleotides to the 3' end of RNA transcribed from the structural nucleic acid coding sequence. The  
20 recombinant vector may further comprise a selectable marker. The selectable marker may generally be any selectable marker suitable for the intended host cell or plant, and preferably is a kanamycin resistance marker, a hygromycin resistance marker, or a herbicide resistance marker. The promoter may be constitutive, inducible, tissue specific, or combinations thereof. The constitutive promoter may generally any constitutive promoter suitable for the  
25 intended host cell or plant, and preferably is CaMV35S, enhanced CaMV35S, FMV, mas, nos, or ocs. The inducible promoter may generally be any inducible promoter suitable for the intended host cell or plant, and preferably is tac, salicylic acid induced, polyacrylic acid induced, safener induced, heat shock promoter, nitrate induced, hormone induced, or light induced. The tissue specific promoter may generally be any tissue specific promoter  
30 suitable for the intended host cell or plant, and preferably is the  $\beta$ -conglycinin 7S promoter,



napin promoter, phaseolin promoter, zein promoter, soybean trypsin inhibitor promoter, ACP promoter, stearyl-ACP desaturase promoter, or oleosin promoter. The nucleic acid sequence encoding a peroxisome targeting protein subunit preferably comprises at least a 6 contiguous nucleic acid sequence from SEQ ID NO:13. The length of the contiguous nucleic acid sequence may be 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, etcetera, 50, 51, 52, etcetera, 100, 101, 102, etcetera, up to and including the entire length of SEQ ID NO:13. The nucleic acid sequence encoding a peroxisome targeting protein subunit is preferably at least 70% identical to SEQ ID NO:13, more preferably at least 80% identical to SEQ ID NO:13, even more preferably at least 90% identical to SEQ ID NO:13, and most preferably is SEQ ID NO:13. The nucleic acid sequence encoding a peroxisome targeting protein subunit preferably hybridizes to SEQ ID NO:13. The encoded peroxisome targeting protein subunit may be an N-terminal or C-terminal peroxisome targeting protein subunit. The encoded N-terminal peroxisome targeting subunit is preferably PTS-2. The encoded C-terminal peroxisome targeting protein subunit preferably comprises a tripeptide. The tripeptide preferably comprises a first amino acid in the N-terminus to C-terminus direction being S, A, or P; a second amino acid in the N-terminus to C-terminus direction being K, R, S, or H; and a third amino acid in the N-terminus to C-terminus direction being L, M, I, or F. The encoded tripeptide preferably is ARM, SRM, SKL, ARL, SRL, PSI, or PRM. The encoded polyhydroxyalkanoate synthase protein subunit is preferably a *Pseudomonas* subunit, and more preferably is a *Pseudomonas aeruginosa* subunit. The nucleic acid sequence encoding a polyhydroxyalkanoate synthase protein subunit preferably comprises at least a 6 contiguous nucleic acid sequence from SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:15, or SEQ ID NO:16. The length of the contiguous nucleic acid sequence may be 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, etcetera, 50, 51, 52, etcetera, 100, 101, 102, etcetera, up to and including the entire length of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:15, or SEQ ID NO:16. The nucleic acid sequence encoding a polyhydroxyalkanoate synthase protein subunit is preferably at least 70% identical to SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:15, or SEQ ID NO:16, more preferably at least 80% identical to SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:15, or SEQ ID NO:16, even more preferably at least 90% identical to SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:15, or SEQ ID NO:16, further preferably is SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:15, or SEQ ID NO:16, and most

preferably is SEQ ID NO:15 or SEQ ID NO:16. The nucleic acid sequence encoding a polyhydroxyalkanoate synthase protein subunit preferably hybridizes to SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:15, or SEQ ID NO:16. The nucleic acid sequence encoding a polyhydroxyalkanoate synthase protein subunit preferably encodes at least a 5 contiguous amino acid sequence from SEQ ID NO:2 or SEQ ID NO:4. The length of the contiguous nucleic acid sequence may be 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, etcetera, 50, 51, 52, etcetera, 100, 101, 102, etcetera, up to and including the entire length of SEQ ID NO:2 or SEQ ID NO:4. The nucleic acid sequence encoding a polyhydroxyalkanoate synthase protein subunit preferably encodes an amino acid sequence at least 70% identical to SEQ ID NO:2 or SEQ ID NO:4, more preferably at least 80% identical to SEQ ID NO:2 or SEQ ID NO:4, even more preferably at least 90% identical to SEQ ID NO:2 or SEQ ID NO:4, and most preferably is SEQ ID NO:2 or SEQ ID NO:4. The structural nucleic acid sequence preferably comprises SEQ ID NO:17 or SEQ ID NO:19, and preferably encodes SEQ ID NO:18 or SEQ ID NO:20.

15 In an alternative embodiment, the invention encompasses a recombinant host cell comprising a nucleic acid segment encoding a non-naturally occurring fusion protein, wherein the nucleic acid segment comprises a nucleic acid sequence encoding a peroxisome targeting protein subunit and a nucleic acid sequence encoding a polyhydroxyalkanoate synthase protein subunit. The recombinant host cell may generally be any type of host cell, and preferably is a fungal or plant host cell. The fungal cell is generally any type of fungal cell, and preferably a *Schizosaccharomyces pombe*, *Streptomyces rimofaciens*, *Fusarium*, *Aspergillus niger*, or *Saccharomyces cerevisiae* cell. The plant cell is generally any type of plant cell, and preferably an alfalfa, banana, barley, bean, cabbage, canola/oilseed rape, carrot, castorbean, celery, clover, coconut, corn, cotton, cucumber, linseed, melon, olive, 20 palm, parsnip, pea, peanut, pepper, potato, potato, radish, rapeseed, rice, soybean, spinach, sunflower, tobacco, tomato, or wheat cell. The recombinant host cell may further comprise a nucleic acid segment encoding an acyl-ACP thioesterase, a fatty acyl hydroxylase, a yeast multifunctional protein (MFP), or an hydroxyacyl-CoA epimerase.

A further alternative embodiment describes a genetically transformed plant cell comprising in the 5' to 3' direction: a) a promoter to direct transcription of a structural nucleic acid sequence encoding a non-naturally occurring fusion protein, wherein the structural nucleic acid sequence comprises: i) a nucleic acid sequence encoding a peroxisome targeting protein subunit; and ii) a nucleic acid sequence encoding a polyhydroxyalkanoate synthase protein subunit; b) a structural nucleic acid sequence encoding a non-naturally occurring fusion protein, wherein the structural nucleic acid sequence comprises: i) a nucleic acid sequence encoding a peroxisome targeting protein subunit; and ii) a nucleic acid sequence encoding a polyhydroxyalkanoate synthase protein subunit; c) a 3' transcription terminator sequence; and d) a 3' polyadenylation signal sequence that directs the addition of polyadenylate nucleotides to the 3' end of RNA transcribed from the structural nucleic acid coding sequence. The plant cell is generally any type of plant cell, and preferably an alfalfa, banana, barley, bean, cabbage, canola/oilseed rape, carrot, castorbean, celery, clover, coconut, corn, cotton, cucumber, linseed, melon, olive, palm, parsnip, pea, peanut, pepper, potato, potato, radish, rapeseed, rice, soybean, spinach, sunflower, tobacco, tomato, or wheat cell. The plant cell may further comprise a nucleic acid segment encoding an acyl-ACP thioesterase, a fatty acyl hydroxylase, a yeast multifunctional protein (MFP), or an hydroxyacyl-CoA epimerase.

An additional embodiment describes a genetically transformed plant comprising in the 5' to 3' direction: a) a promoter to direct transcription of a structural nucleic acid sequence encoding a non-naturally occurring fusion protein, wherein the structural nucleic acid sequence comprises: i) a nucleic acid sequence encoding a peroxisome targeting protein subunit; and ii) a nucleic acid sequence encoding a polyhydroxyalkanoate synthase protein subunit; b) a structural nucleic acid sequence encoding a non-naturally occurring fusion protein, wherein the structural nucleic acid sequence comprises: i) a nucleic acid sequence encoding a peroxisome targeting protein subunit; and ii) a nucleic acid sequence encoding a polyhydroxyalkanoate synthase protein subunit; c) a 3' transcription terminator sequence; and d) a 3' polyadenylation signal sequence that directs the addition of polyadenylate nucleotides to the 3' end of RNA transcribed from the structural nucleic acid coding sequence. The plant may generally be any type of plant, and preferably an alfalfa, banana,

barley, bean, cabbage, canola/oilseed rape, carrot, castorbean, celery, clover, coconut, corn, cotton, cucumber, linseed, melon, olive, palm, parsnip, pea, peanut, pepper, potato, potato, radish, rapeseed, rice, soybean, spinach, sunflower, tobacco, tomato, or wheat plant. The promoter may be constitutive, inducible, tissue specific, or combinations thereof. The  
5 constitutive promoter may generally any constitutive promoter suitable for the intended plant, and preferably is CaMV35S, enhanced CaMV35S, FMV, mas, nos, or ocs. The inducible promoter may generally be any inducible promoter suitable for the intended plant, and preferably is tac, salicylic acid induced, polyacrylic acid induced, safener induced, heat shock promoter, nitrate induced, hormone induced, or light induced. The tissue specific  
10 promoter is generally any tissue specific promoter, and preferably is the  $\beta$ -conglycinin 7S promoter, napin promoter, phaseolin promoter, zein promoter, soybean trypsin inhibitor promoter, ACP promoter, stearoyl-ACP desaturase promoter, or oleosin promoter. The plant may further comprise a nucleic acid segment encoding an acyl-ACP thioesterase, a fatty acyl hydroxylase, a yeast multifunctional protein (MFP), or an hydroxyacyl-CoA  
15 epimerase.

The invention describes a method for preparing host cells useful to produce a non-naturally occurring fusion protein comprising the steps of: a) selecting a host cell b) transforming the selected host cell with a recombinant vector having a structural nucleic acid sequence encoding a non-naturally occurring fusion protein, wherein the structural  
20 nucleic acid sequence comprises: i) a nucleic acid sequence encoding a peroxisome targeting protein subunit; and ii) a nucleic acid sequence encoding a polyhydroxyalkanoate synthase protein subunit; and c) obtaining transformed host cells. The vector may further comprise a selectable marker. The selectable marker may generally be any selectable marker suitable for use in the intended host cell, and more preferably for plants is a  
25 kanamycin resistance marker, a hygromycin resistance marker, or a herbicide resistance marker. The host cell may generally be any type of cell, and preferably is a fungal or plant cell. The fungal cell may generally be any type of fungal cell, and more preferably is a *Schizosaccharomyces pombe*, *Streptomyces rimofaciens*, *Fusarium*, *Aspergillus niger*, or *Saccharomyces cerevisiae* cell. The plant cell may generally be any type of plant cell, and  
30 more preferably is an alfalfa, banana, barley, bean, cabbage, canola/oilseed rape, carrot,

castorbean, celery, clover, coconut, corn, cotton, cucumber, linseed, melon, olive, palm, parsnip, pea, peanut, pepper, potato, potato, radish, rapeseed, rice, soybean, spinach, sunflower, tobacco, tomato, or wheat cell.

The invention further describes a method of preparing a transformed plant useful to produce a non-naturally occurring fusion protein comprising the steps of: a) selecting a host plant cell b) transforming the selected host cell with a recombinant vector having a structural nucleic acid sequence encoding a non-naturally occurring fusion protein, wherein the structural nucleic acid sequence comprises: i) a nucleic acid sequence encoding a peroxisome targeting protein subunit; and ii) a nucleic acid sequence encoding a polyhydroxyalkanoate synthase protein subunit; c) obtaining transformed host plant cells; and d) regenerating the transformed host plant cells. The vector may further comprise a selectable marker. The selectable marker may generally be any selectable marker suitable for use in the intended host cell, and more preferably is a kanamycin resistance marker, a hygromycin resistance marker, or a herbicide resistance marker. The host plant cell may generally be any type of plant cell, and more preferably is an alfalfa, banana, barley, bean, cabbage, canola/oilseed rape, carrot, castorbean, celery, clover, coconut, corn, cotton, cucumber, linseed, melon, olive, palm, parsnip, pea, peanut, pepper, potato, potato, radish, rapeseed, rice, soybean, spinach, sunflower, tobacco, tomato, or wheat cell. The invention also encompasses the plant made by the above described methods.

A preferred embodiment is a method for the preparation of a polyhydroxyalkanoate, comprising the steps of: a) obtaining a cell capable of producing a non-naturally occurring fusion protein, wherein the fusion protein comprises: i) a peroxisome targeting protein subunit; and ii) a polyhydroxyalkanoate synthase protein subunit; b) establishing a culture of the cell; and c) culturing the cell under conditions suitable for the production of the polyester. The method may further comprise isolating the polyhydroxyalkanoate from the cultured cell. The culture may further comprise fatty acids, and more preferably natural fatty acids, non-natural or synthetic fatty acids, or mixtures thereof. The cell may generally be any type of cell, and preferably is a fungal or plant cell. The fungal cell may generally be any type of fungal cell, and more preferably is a *Schizosaccharomyces pombe*, *Streptomyces*

*rimofaciens*, *Fusarium*, *Aspergillus niger*, or *Saccharomyces cerevisiae* cell. The plant cell may generally be any type of plant cell, and more preferably is an alfalfa, banana, barley, bean, cabbage, canola/oilseed rape, carrot, castorbean, celery, clover, coconut, corn, cotton, cucumber, linseed, melon, olive, palm, parsnip, pea, peanut, pepper, potato, potato, radish, rapeseed, rice, soybean, spinach, sunflower, tobacco, tomato, or wheat cell. The polyhydroxyalkanoate isolated from the cell may generally be any type of polyhydroxyalkanoate, and preferably comprises 3-hydroxyhexanoic acid (H:6), 3-hydroxyoctanoic acid (H:8), 3-hydroxydecanoic acid (H:10), 3-hydroxydodecanoic acid (H:12), 3-hydroxytetradecanoic acid (H:14), 3-hydroxyhexadecanoic acid (H:16), 3-hydroxyheptanoic acid (H:7), 3-hydroxynonanoic acid (H:9), 3-hydroxyundecanoic acid (H:11), 3-hydroxytridecanoic acid (H:13), 3-hydroxyhexadecatrienoic acid (H16:3), 3-hydroxyhexadecadienoic acid (H16:2), 3-hydroxyhexadecenoic acid (H16:1), 3-hydroxytetradecatrienoic acid (H14:3), 3-hydroxytetradecadienoic acid (H14:2), 3-hydroxytetradecenoic acid (H14:1), 3-hydroxydodecadienoic acid (H12:2), 3-hydroxydodecenoic acid (H12:1), 3-hydroxyoctenoic acid (H8:1), 4-hydroxydecanoic acid, 8-methyl-3-hydroxynonanoic acid, or 6-methyl-3-hydroxyheptanoic acid monomers.

In a further preferred embodiment, the invention presents a method for the preparation of a polyhydroxyalkanoate, comprising the steps of: a) obtaining a plant capable of producing a non-naturally occurring fusion protein, wherein the fusion protein comprises: i) a peroxisome targeting protein subunit; and ii) a polyhydroxyalkanoate synthase protein subunit; and c) growing the plant under conditions suitable for the production of the polyhydroxyalkanoate. The method may further comprise the step of isolating the polyhydroxyalkanoate from the plant. The method may further comprise supplementing the plant with natural fatty acids, non-natural fatty acids, or mixtures thereof. The plant may generally be any type of plant, and preferably is an alfalfa, banana, barley, bean, cabbage, canola/oilseed rape, carrot, castorbean, celery, clover, coconut, corn, cotton, cucumber, linseed, melon, olive, palm, parsnip, pea, peanut, pepper, potato, potato, radish, rapeseed, rice, soybean, spinach, sunflower, tobacco, tomato, or wheat plant. The polyhydroxyalkanoate isolated from the plant may generally be any type of polyhydroxyalkanoate, and preferably comprises 3-hydroxyhexanoic acid (H:6), 3-

hydroxyoctanoic acid (H:8), 3-hydroxydecanoic acid (H:10), 3-hydroxydodecanoic acid (H:12), 3-hydroxytetradecanoic acid (H:14), 3-hydroxyhexadecanoic acid (H:16), 3-hydroxyheptanoic acid (H:7), 3-hydroxynonanoic acid (H9), 3-hydroxyundecanoic acid (H:11), 3-hydroxytridecanoic acid (H:13), 3-hydroxyhexadecatrienoic acid (H16:3), 3-hydroxyhexadecadienoic acid (H16:2), 3-hydroxyhexadecenoic acid (H16:1), 3-hydroxytetradecatrienoic acid (H14:3), 3-hydroxytetradecadienoic acid (H14:2), 3-hydroxytetradecenoic acid (H14:1), 3-hydroxydodecadienoic acid (H12:2), 3-hydroxydodecenoic acid (H12:1), 3-hydroxyoctenoic acid (H8:1), 4-hydroxydecanoic acid, 8-methyl-3-hydroxynonanoic acid, or 6-methyl-3-hydroxyheptanoic acid monomers.

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The invention further encompasses plants containing polyhydroxyalkanoates, wherein the polyhydroxyalkanoate comprises 3-hydroxyhexanoic acid (H:6), 3-hydroxyoctanoic acid (H:8), 3-hydroxydecanoic acid (H:10), 3-hydroxydodecanoic acid (H:12), 3-hydroxytetradecanoic acid (H:14), 3-hydroxyhexadecanoic acid (H:16), 3-hydroxyheptanoic acid (H:7), 3-hydroxynonanoic acid (H9), 3-hydroxyundecanoic acid (H:11), 3-hydroxytridecanoic acid (H:13), 3-hydroxyhexadecatrienoic acid (H16:3), 3-hydroxyhexadecadienoic acid (H16:2), 3-hydroxyhexadecenoic acid (H16:1), 3-hydroxytetradecatrienoic acid (H14:3), 3-hydroxytetradecadienoic acid (H14:2), 3-hydroxytetradecenoic acid (H14:1), 3-hydroxydodecadienoic acid (H12:2), 3-hydroxydodecenoic acid (H12:1), 3-hydroxyoctenoic acid (H8:1), 4-hydroxydecanoic acid, 8-methyl-3-hydroxynonanoic acid, or 6-methyl-3-hydroxyheptanoic acid monomers.

20

In an alternative embodiment, the invention describes polyhydroxyalkanoates comprising 3-hydroxyhexadecatrienoic acid (H16:3), 3-hydroxyhexadecadienoic acid (H16:2), 3-hydroxytetradecatrienoic acid (H14:3), or 3-hydroxydodecadienoic acid (H12:2) monomers.

25

## DESCRIPTION OF THE FIGURES

The following figure forms part of the present specification and is included to further demonstrate certain aspects of the present invention. The invention may be better

understood by reference to the figure in combination with the detailed description of specific embodiments presented herein.

Figure 1: GC-MS analysis of PHA in transgenic plants. Trans-esterified chloroform extracts from phaC1-transformed line 3.3 (1A, 1B) and vector-transformed line 21 (1C, 1D) were analyzed. In panels 1A and 1C, the total ion chromatogram is presented, while on panel 1B and 1D, only ions with a mass-to-charge ratio of 103 are shown.

### DESCRIPTION OF THE SEQUENCE LISTINGS

The following sequence listings form part of the present specification and are included to further demonstrate certain aspects of the present invention. The invention may be better understood by reference to one or more of these sequence listings in combination with the detailed description of specific embodiments presented herein.

SEQ ID NO:1	Wild type PHA synthase C1 nucleic acid sequence.
SEQ ID NO:2	Wild type PHA synthase C1 protein sequence.
SEQ ID NO:3	Wild type PHA synthase C2 nucleic acid sequence.
SEQ ID NO:4	Wild type PHA synthase C2 protein sequence.
SEQ ID NO:5	Forward PCR primer for PHA synthase C1 fusion sequence.
SEQ ID NO:6	Reverse PCR primer for PHA synthase C1 fusion sequence.
SEQ ID NO:7	Forward PCR primer for PHA synthase C2 fusion sequence.
SEQ ID NO:8	Reverse PCR primer for PHA synthase C2 fusion sequence.
SEQ ID NO:9	Wild type isocitrate lyase nucleic acid sequence.
SEQ ID NO:10	Wild type isocitrate lyase protein sequence.
SEQ ID NO:11	Forward PCR primer for isocitrate lyase fusion sequence.
SEQ ID NO:12	Reverse PCR primer for isocitrate lyase fusion sequence.
SEQ ID NO:13	Nucleic acid sequence encoding the isocitrate lyase peroxisome targeting protein subunit.
SEQ ID NO:14	Isocitrate lyase peroxisome targeting protein subunit.
SEQ ID NO:15	PHA synthase C1 nucleic acid sequence with plant preferred codon.



- SEQ ID NO:16 PHA synthase C2 nucleic acid sequence with plant preferred codon.
- SEQ ID NO:17 Nucleic acid sequence encoding PHA synthase C1 and isocitrate lyase fusion protein.
- 5 SEQ ID NO:18 PHA synthase C1 and isocitrate lyase fusion protein.
- SEQ ID NO:19 Nucleic acid sequence encoding PHA synthase C2 and isocitrate lyase fusion protein.
- SEQ ID NO:20 PHA synthase C2 and isocitrate lyase fusion protein.
- SEQ ID NO:21 PCR amplified nucleic acid sequence encoding wild type *Candida albicans* MFP.
- 10 SEQ ID NO:22 Wild type *Candida albicans* MFP protein.
- SEQ ID NO:23 PCR amplified nucleic acid sequence encoding SKL mutant *Candida albicans* MFP.
- SEQ ID NO:24 *Candida albicans* MFP protein with SKL substitution for AKI.
- 15 SEQ ID NO:25 PCR amplified nucleic acid sequence encoding mutant *Candida albicans* MFP lacking AKI sequence.
- SEQ ID NO:26 *Candida albicans* MFP protein lacking AKI sequence.

## DEFINITIONS

20 The following definitions are provided in order to aid those skilled in the art in understanding the detailed description of the present invention.

"Acyl-ACP thioesterase" refers to proteins which catalyze the hydrolysis of acyl-ACP thioesters.

"C-terminal region" refers to the region of a peptide, polypeptide, or protein chain  
25 from the middle thereof to the end that carries the amino acid having a free a carboxyl group (the C-terminus).

"CoA" refers to coenzyme A.

The phrases "coding sequence", "open reading frame", and "structural sequence" refer to the region of continuous sequential nucleic acid triplets encoding a protein, polypeptide, or peptide sequence.

5       The term "encoding DNA" or "encoding nucleic acid" refers to chromosomal nucleic acid, plasmid nucleic acid, cDNA, or synthetic nucleic acid which codes on expression for any of the proteins or fusion proteins discussed herein.

"Fatty acyl hydroxylase" refers to proteins which catalyze the conversion of fatty acids to hydroxylated fatty acids.

10       The term "gene" refers to chromosomal DNA, plasmid DNA, cDNA, synthetic DNA, or other DNA that encodes a peptide, polypeptide, protein, or RNA molecule, and regions flanking the coding sequence involved in the regulation of expression.

15       The term "genome" as it applies to bacteria encompasses both the chromosome and plasmids within a bacterial host cell. Encoding DNAs of the present invention introduced into bacterial host cells can therefore be either chromosomally-integrated or plasmid-localized. The term "genome" as it applies to plant cells encompasses not only chromosomal DNA found within the nucleus, but organelle DNA found within subcellular components of the cell. DNAs of the present invention introduced into plant cells can therefore be either chromosomally-integrated or organelle-localized.

20       "Glyoxysome" and "peroxisome" refer to the same organelle in a plant. Glyoxysome refers to a type of peroxisome found in germinating seedlings, senescing tissues, or in dark-grown tissues. Glyoxysomes and peroxisomes contain enzymes responsible for the conversion of lipids to carbohydrates.

25       "Identity" refers to the degree of similarity between two nucleic acid or protein sequences. An alignment of the two sequences is performed by a suitable computer

program. A widely used and accepted computer program for performing sequence alignments is CLUSTALW v1.6 (Thompson, et al. *Nucl. Acids Res.*, 22: 4673-4680 (1994)). The number of matching bases or amino acids is divided by the total number of bases or amino acids, and multiplied by 100 to obtain a percent identity. For example, if two 580 base pair sequences had 145 matched bases, they would be 25 percent identical. If the two compared sequences are of different lengths, the number of matches is divided by the shorter of the two lengths. For example, if there were 100 matched amino acids between 200 and a 400 amino acid proteins, they are 50 percent identical with respect to the shorter sequence.

10       The terms "microbe" or "microorganism" refer to algae, bacteria, fungi, and protozoa.

"N-terminal region" refers to the region of a peptide, polypeptide, or protein chain from the amino acid having a free amino group to the middle of the chain.

"Nucleic acid" refers to ribonucleic acid (RNA) and deoxyribonucleic acid (DNA).

15       A "nucleic acid segment" is a nucleic acid molecule that has been isolated free of total genomic DNA of a particular species, or that has been synthesized. Included with the term "nucleic acid segment" are DNA segments, recombinant vectors, plasmids, cosmids, phagemids, phage, viruses, etcetera.

20       "Overexpression" refers to the expression of a polypeptide or protein encoded by a DNA introduced into a host cell, wherein said polypeptide or protein is either not normally present in the host cell, or wherein said polypeptide or protein is present in said host cell at a higher level than that normally expressed from the endogenous gene encoding said polypeptide or protein.

25       The term "plastid" refers to the class of plant cell organelles that includes amyloplasts, chloroplasts, chromoplasts, elaioplasts, coplasts, etioplasts, leucoplasts, and proplastids. These organelles are self-replicating, and contain what is commonly referred to

as the "chloroplast genome," a circular DNA molecule that ranges in size from about 120 to about 217 kb, depending upon the plant species, and which usually contains an inverted repeat region (Fosket, Plant growth and Development, Academic Press, Inc., San Diego, CA, p. 132 (1994)).

5        "Polyadenylation signal" or "polyA signal" refers to a nucleic acid sequence located 3' to a coding region that directs the addition of adenylate nucleotides to the 3' end of the mRNA transcribed from the coding region.

The term "polyhydroxyalkanoate (or PHA) synthase" refers to enzymes that convert hydroxyacyl-CoAs to polyhydroxyalkanoates and free CoA.

10        The term "promoter" or "promoter region" refers to a nucleic acid sequence, usually found upstream (5') to a coding sequence, that controls expression of the coding sequence by controlling production of messenger RNA (mRNA) by providing the recognition site for RNA polymerase and/or other factors necessary for start of transcription at the correct site. As contemplated herein, a promoter or promoter region includes variations of promoters  
15        derived by means of ligation to various regulatory sequences, random or controlled mutagenesis, and addition or duplication of enhancer sequences. The promoter region disclosed herein, and biologically functional equivalents thereof, are responsible for driving the transcription of coding sequences under their control when introduced into a host as part of a suitable recombinant vector, as demonstrated by its ability to produce mRNA.

20        "Protein subunit" refers to a protein sequence that is part of a fusion protein. Examples are  $\beta$ -galactosidase, FLAG, green fluorescent protein, and in the instant invention, polyhydroxyalkanoate synthase, and a peroxisome or glyoxysome targeting peptide.

25        "PTS2" refers to an N-terminal protein subunit having the sequence (R/K)(L/Q/I)XXXXXX(H/Q)L, wherein X is any amino acid.

“Regeneration” refers to the process of growing a plant from a plant cell (e.g., plant protoplast or explant).

“Transformation” refers to a process of introducing an exogenous nucleic acid sequence (e.g., a vector, recombinant nucleic acid molecule) into a cell or protoplast in which that exogenous nucleic acid is incorporated into a chromosome or is capable of autonomous replication.

A “transformed cell” or “transgenic cell” is a cell whose DNA has been altered by the introduction of an exogenous nucleic acid molecule into that cell.

A “transformed plant” or “transgenic plant” is a plant whose DNA has been altered by the introduction of an exogenous nucleic acid molecule into that plant, or by the introduction of an exogenous nucleic acid molecule into a plant cell from which the plant was regenerated or derived.

The following examples are included to demonstrate preferred embodiments of the invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples which follow represent techniques discovered by the inventors to function well in the practice of the invention, and thus can be considered to constitute preferred modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

## EXAMPLES

### EXAMPLE 1: Plant material

*Arabidopsis thaliana*, race Columbia, was transformed by the vacuum infiltration method (Bechtold, N., et al., *C.R. Acad. Sci. Paris* 316: 1194-1199 (1993)). Transformants

were selected on media containing Murashige and Skoog salts ("MS", Murashige, T. and Skoog, F., *Physiol. Plant.* 15: 473-497 (1962)), 1% (w/v) sucrose, 0.7% (w/v) agar and 50 µg/mL kanamycin. Kanamycin-resistant plants were subsequently transferred to soil and grown under continuous fluorescent light at 19°C. In some experiments, plants were grown  
5 under constant agitation (100 rpm) for 1-2 weeks in liquid media containing MS salts and 2% sucrose.

#### EXAMPLE 2: Cloning of peroxisomally targeted PHA synthases C1 and C2

The *phaC1* and *phaC2* genes were obtained from Steinbüchel (Timm, A. and Steinbüchel, A., *Eur. J. Biochem.* 209: 14-30 (1992), GenBank Accession Number  
10 X66592). PCR was used to amplify the genes and to modify their 5'- and 3'-termini as follows: At the 5'-end the codons encoding the serine-2 and the arginine-2 residue of *phaC1* and *phaC2*, respectively, were modified to conform more closely with the general codon preferences of *A. thaliana* (Meyerowitz, E. M. in *Methods in Arabidopsis research*, eds. Koncz, C., Chua, N.-H. & Schell, J. (World Scientific Publishing, Singapore), pp. 100-  
15 119 (1992)). At the 3'-end the sequences were modified to obtain suitable cloning sites and to delete the stop codons to enable the construction of chimerical fusions with the peroxisomal targeting sequence.

The carboxy-terminal 35 amino acid residues of the isocitrate lyase gene (ICL) (Olsen, L.J., et al., *Plant Cell* 5: 941-952 (1993), GenBank Accession Number Y13356)  
20 from *Brassica napus* were used as targeting sequence for the PHA synthases C1 and C2. It has been shown previously that this sequence was sufficient to ensure the peroxisomal localization of the chloramphenicol acetyl transferase (CAT) to the peroxisomes in *A. thaliana* (Comai, L. et al., *The Plant Cell* 1: 293-300 (1989); Olsen, L. J. et al., *The Plant Cell* 5: 941-952 (1993); Zhang, J. Z. et al., *Mol. Gen. Genet.* 238: 177-184 (1993)). A PCR  
25 product encoding the ICL targeting sequence was cloned into the vector pART7 (Gleaves, A.P., *Plant Mol. Biol.* 20: 1202-1207 (1992), GenBank Accession Number X69707). The PCR products containing the *phaC1* or *phaC2* genes were cloned 5'-upstream of the ICL sequence to produce a contiguous open reading frame encoding the targeted fusion proteins.

The 5'- and 3'-ends of the genes in the resulting plasmids pART7\_phaC1\_ICL and pART7\_phaC2\_ICL were sequenced to verify the modifications.

The PHA accumulation-deficient mutant *Pseudomonas putida* KT2440 NK2:3 was obtained from Steinbüchel for complementation studies to verify the enzyme activities of the modified PHA synthases C1 and C2. The *phaC1\_ICL* and *phaC2\_ICL* genes were cloned into the broad-host range plasmid pVLT35 behind the IPTG-inducible tac-promoter (Lorenzo, V. et al., *Gene* 123: 17-24 (1993)) and electroporated into the *P. putida* mutant. Streptomycin-resistant transformants were subcultured onto minimal medium containing either octanoate or gluconate as sole carbon source. The Nile Blue A fluorescence stain (Page, W. J. and C. J. Tenove, *Biotechnology Techniques* 10: 215-220 (1996)) was used to visualize PHA accumulation. Upon IPTG induction PHA accumulation was observed with pVLT35\_phaC1\_ICL and pVLT35\_phaC2\_ICL, but not with pVLT35 alone, thus indicating that the modified genes were still active.

### EXAMPLE 3: Plant transformation and screening for PHA synthase C1 transgenic plants

The NotI-cassettes of plasmids pART7\_phaC1\_ICL and pART7\_phaC2\_ICL containing the modified genes flanked by the Cauliflower mosaic virus 35S promoter (CaMV35S) and the octopine synthase (ocs) 3'-terminator were cloned into the plant binary vector pART27 to obtain pART27\_phaC1\_ICL and pART27\_phaC2\_ICL. These plasmids were transformed into *A. thaliana* ecotype Columbia by *Agrobacterium* GV3101-mediated transfer utilizing an *in planta* vacuum-infiltration method (Bechtold, N. et al., *C.R. Acad. Sci. Paris* 316: 1194-1199 (1993)). Transgenic T1 plants were selected for antibiotic resistance during germination of the seeds of infiltrated plants on plant growth medium containing mineral salts, sucrose and kanamycin. Negative control plants containing only the insert-less T-DNA of the vector pART27 were obtained in the same way.

Transgenic PHAC1 plants (T1) expressing high amounts of PHA synthase C1 were selected by Western analysis with an antiserum against the PHA synthase C1, which was

obtained from Steinbüchel's laboratory. Unfortunately no antibodies against PHA synthase C2 were found to be suitable, so a different screening strategy was used, see below. Six independent lines expressing varying quantities of PHA synthase C1 were obtained from 12 originally infiltrated plants, which had been harvested individually (another 19 have not yet been investigated). Initially some problems with the western analysis were encountered, one of which was the precipitation of the PHA synthase in plant protein extracts upon freezing. Analysis of the kanamycin segregation of the second generation (T2) and third generation (T3) plants indicated that three of these lines contained multilocus T-DNA inserts. Initially these lines exhibited the highest expression of PHA synthase C1 as judged by western analysis, however, the expression of the transgene in these lines was variable in plants of the T2 and T3 generation and complete "silencing" was observed. The line PHAC1#3.3 was finally chosen for further studies, because it contained a single-locus T-DNA insert and exhibited stable expression of the transgene as seen on the western blot.

#### EXAMPLE 4: PHA production by PHAC1 plants

A protocol for the detection of monomers of PHA by gas chromatography was developed based on the method described for the extraction of PHB from *Arabidopsis* (Poirier, Y. et al., *Int. J. Biol. Macromol.* 17: 7-12 (1995)). Whole leaves were extracted several times with ethanol and methanol to elute all the soluble lipids, thereafter chloroform and methanol acidified with 3% (v/v) H<sub>2</sub>SO<sub>4</sub> were added in equal volumes and the reactions were put at 98°C for 4 hours to transesterify the PHA polyester. GC-chromatograms of the resulting chloroform extracts showed a large number of peaks, most of which were due to the derivatization of various leave compounds. Peaks corresponding to the standards of the expected methyl esters of PHA monomers were, however, distinguishable amongst the others. A large fraction of the plant material was solubilized during this transesterification treatment, it was however not determined whether underivatized PHA remained in the solid underivatized material. This made the quantification of the PHA in plant material slightly uncertain, but the authors estimated intuitively that most of the PHA in the material became derivatized preferentially. The GC-standards (from Sigma Chemical, St. Louis, MO, except H6 which was from Beat Keller) were the methyl esters of D-3-hydroxy-hexanoic acid (3-



OH-caproic acid, H6 monomer), DL-3-hydroxy-octanoic acid (3-OH-caprylic acid, H8 monomer), DL-3-hydroxy-capric acid (H10 monomer), DL-3-hydroxy-lauric acid (H12 monomer) and DL-3-hydroxy-myristic acid (H14 monomer).

The transgenic plants expressing the PHA synthase C1 showed a significant increase in the size of the peaks corresponding to the H6-H14 monomers compared to the negative control plants. One novel peak was found only in PHAC1 plants and never in the negative controls. GC-MS was used to confirm that the peaks observed in both the PHAC1 plants and the negative controls were really identical to the standards and the novel peak was determined as being due to 3-hydroxy-octenoyl-methyl-ester containing a single unsaturated bond (H8:1 monomer). It is being speculated that the unsaturated bond is located at carbon 5 and has the cis conformation and that this monomer is due to the degradation of  $\alpha$ -linolenic acid (18:3, all-cis,  $\Delta$ 9,12,15) and 16:3 (all-cis,  $\Delta$ 7, 10, 13) by  $\beta$ -oxidation. This reasoning is based on the prediction, that a D-3-hydroxy-octenoyl-CoA  $\beta$ -oxidation intermediate arises due to the cis-double bond at the even-numbered carbons (Gerhardt, B., *Lipid metabolism in plants* (Moore, T. S., Jr., ed.), CRC Press Inc., pp. 527-565 (1993)); see further discussions below under feeding studies). The same argument can be taken for the generation of the other monomers incorporated into the PHA, i.e. that they originated from fatty acids having a double bond at even-numbered carbons, which resulted in the formation of D-3-hydroxy-acyl-CoA  $\beta$ -oxidation intermediates. Thus the H8 monomer would originate from the degradation of linoleic acid (C18:2, all-cis,  $\Delta$ 9,12) or from C16:2, all-cis,  $\Delta$ 7, 10. This however does not satisfactorily explain the whole range of monomers observed, e.g. the H6 monomer would then have to originate from the fatty acids C18:1,  $\Delta$ 14-cis or C16:1,  $\Delta$ 12-cis, while the H14 monomer would have to originate from C18:1,  $\Delta$ 8-cis, or C16:1,  $\Delta$ 4-cis or C14:1,  $\Delta$ 2-cis, etcetera. As most of these would be rather uncommon fatty acids in *A. thaliana*, another argument for the origin of these PHA monomers can be proposed, which is based on the existence of an epimerase activity in plant  $\beta$ -oxidation (Preisig-Müller, R. et al., *J. Biol. Chem.* 269: 20475-20481 (1994)). In this case the D-3-OH-acyl-CoA  $\beta$ -oxidation intermediates are generated at a low rate by the "reverse" reaction catalyzed by the epimerase required for the conversion of D-3-hydroxy-acyl-CoA to the L-form, and sequestration of these D-intermediates into PHA actually

drives the reverse reaction. In this way the whole range of possible monomers can be explained, while the argument involving the unsaturated bond at even-numbered carbons in the acyl chains would still explain the relatively higher proportion of the H8-monomer and the existence of the H8:1 monomer.

5        Several negative control plants (both *A. thaliana* wild type and pART27 transgenic plants) were analyzed in various experiments without ever seeing more than only trace amounts of the various saturated monomers. The concentrations present in the negative controls were at least 1000 times smaller than in the positive plants, close to the detection limit of the methods at our availability. This was done by utilizing the GC-MS in the SIM  
10    mode (selected ion monitoring; ion 103 is characteristic for all of these 3-OH-fatty acid methyl esters) for which the detection limit was found to be approximately 4 pg/ $\mu$ L of the various standards. These compounds in the negative controls might also be intermediates of  $\beta$ -oxidation, i.e. mostly the L-3-hydroxy-acyl-CoAs and perhaps even very low amounts of the D-form, which are normally present at very low concentrations in the plant material in  
15    which  $\beta$ -oxidation is taking place. A rough calculation indicated a total PHA content of 0.03% (w/dry weight) in PHAC1#4.4 (multilocus plant), which related to approximately 5  $\mu$ g of PHA in a large fresh leave weighing 155 mg. It was approximated that line PHAC1#3.3 produced 0.01% (weight/dry weight) in soil-grown plants.

#### EXAMPLE 5: Screening for PHA synthase C2 expressing plants

20        PHAC2 plants were screened directly for PHA production by analysis of dry leaves of T2 plants. Almost all of the T2 plants derived from 13 independently transformed plants were found to produce PHA in varying quantities, as judged by the presence of the novel peak due to the C8:1 monomer and also the peaks of the other PHA monomers. The highest producing plants were analyzed further and homozygous T3 plants were obtained. Two  
25    homozygous single-locus T3 lines were selected, PHAC2#19.5 and PHAC2#8.6. In comparison to PHAC1#3.3 plants, these PHAC2 plants produced slightly smaller quantities of PHA in seedlings grown on plates containing MS salts, kanamycin and sucrose. The

monomer composition of the respective transgenic plants was however identical. For that reason most of the further studies were only done with line PHAC1#3.3.

**EXAMPLE 6: Immunolocalization and observation of PHA granules**

5 For the immunolocalization of the peroxisomally-targeted PHA synthase C1, T3 seedlings of lines PHAC1#3.3 and pART27#21 (negative control) were grown on plates containing MS salts, kanamycin and sucrose. Seedlings were grown for 7 days under continuous light or in the dark after one day of illumination, the latter was done to obtain etiolated seedlings in which glyoxysomes are more abundant. The seedlings were fixed and  
10 sent together with some anti-PHA synthase C1 antiserum to Prof. Leech's laboratory at the University of York, where the immunolocalization was performed. It was found that the peroxisomes in PHAC1 seedlings were initially difficult to identify, since they did not look normal due to the presence of granules within them. These granules were very abundant in the etiolated seedlings, while in the light-grown seedlings most of the peroxisomes still  
15 looked normal or seemed to contain only tiny granules. The PHA synthase C1 was located in what seem to be two different types of organelles or peroxisomes, because the one contains a large quantity of PHA granules while the other contains apparently none. The darker peroxisomes without granules corresponded in appearance most closely to the normal peroxisomes in the negative controls. It is possible that this apparent heterogeneity is simply  
20 the results of non-homogenous distribution of granules within the peroxisomes. Glycolate oxidase was used as marker enzyme for peroxisomes of seedlings grown under light, while rubisco was used as chloroplastic marker. Antibodies against these two marker enzymes clearly identified the respective organelles in both PHAC1 seedlings and in the pART27 negative controls. Glycolate oxidase was found to be located in the organelles, i.e. the  
25 peroxisomes, containing PHA granules. Similarly the enzyme isocitrate lyase (ICL) was used as glyoxysomal marker in etiolated seedlings and it also confirmed that the granule-containing organelles were glyoxysomes. The antiserum against PHA synthase C1 unambiguously identified the peroxisomal localization of the PHA synthase in the PHAC1 seedlings, while it did not detect anything in the negative controls. Unusual accumulations  
30 of granules were also observed occasionally in the vacuoles of etiolated PHAC1 seedlings

and these globules were gold-labelled with anti-PHA synthase C1. This was in correspondence with the observation that the PHB synthase is found on the surface of PHB granules in bacteria (Gerngross, T. U. et al., *J. Bacteriol.* 175, 5289-5293 (1993)).

EXAMPLE 7: Changing PHA yield and monomer composition in feeding studies

5 Line PHAC1#3.3 was used to investigate if the total yield of PHA could be increased or if PHAs containing other monomers than the "native" PHA could be synthesized in PHAC1 transgenic plants. For that purpose seeds were sterilized and germinated in liquid medium containing mineral salts and 2% (w/v) sucrose supplemented  
10 with fatty acids or other compounds known to be degraded by  $\beta$ -oxidation. In experiment #1 the seedlings were grown for 3 days in the light before the substrates were added and the plant were moved into the dark. The material was harvested after 8 days and derivatized samples were analyzed by gas chromatography.

The results summarized in Table 1 point out several encouraging aspects. The yield of native PHA (obtained without feeding any substrate) was doubled when seedlings were  
15 germinated in the dark as opposed to continuous illumination. This could perhaps be ascribed to a more complete mobilization of the seed lipids in etiolated seedlings. In this respect the regulation of the glyoxylate cycle enzymes malate synthase and isocitrate lyase might play a role by affecting lipid-mobilization via  $\beta$ -oxidation. It has been shown that these glyoxylate cycle enzymes are regulated transcriptionally by three types of signal,  
20 namely light regulation, carbon catabolite repression by various sugars and developmental regulation during germination and senescence (Graham, I. A. et al., *Plant Mol. Biol.* 15: 539-549 (1990); Graham, I. A. et al., *Plant Cell* 4:349-357 (1992); Graham, I. A. et al., *Plant Cell* 6: 761-772 (1994)).

The large increase in the PHA yield obtained by the feeding of TWEEN-20 (Sigma;  
25 50% palmitic acid (C16) esterified with polyoxyethylenesorbitol, the remainder is made up by lauric acid (C12) and myristic acid (C14) also esterified) (TWEEN is a registered trademark of ICI Americas, Inc., Wilmington, DE) indicated that the PHA synthase was

very active in these plants and thus not responsible for the relatively low yield of native PHA in seedlings grown without added fatty acids. The most pronounced effect of TWEEN-20 on the monomer composition was the decrease in the content of the H8:1 monomer from about 30% in native PHA to about 1%, which was most likely due to the lack of unsaturated fatty acid derivatives in the TWEEN-20. The relative distribution of the other monomers could be explained by the step-by-step  $\beta$ -oxidation of the C16, C14 and C12 components in TWEEN-20. A negative effect on seedling growth due to TWEEN-20 was observed, but it was small considering its high concentration (5% v/v) in the medium.

The accumulation of PHA granules in PHAC1 seedlings grown in liquid cultures supplemented with 5% TWEEN-20 under constant illumination for 12 days was very striking on electron microscope micrographs. These PHA granules were not observed in the negative controls, i.e. pART27 transgenic seedlings fed with TWEEN-20. The granules looked different from the starch granules observed in chloroplasts. These electron microscopic studies were done in our own institute by Mrs J. Petétot and the results confirmed similar results obtained with etiolated seedlings in Prof. Leech's laboratory.

TWEEN-60 (Sigma; 50% stearic acid (C18) and some palmitic and myristic acid; all esterified to polyoxyethylenesorbitol) and TWEEN-80 (Sigma; 50% oleic acid (C18:1), esterified to polyoxyethylenesorbitol) had less impact on the PHA yield, the monomer composition and the seedling growth than TWEEN-20. The relatively high level of the H8:1 monomer might be due to a higher contamination of TWEEN-60 and -80 with unsaturated fatty acids like  $\alpha$ -linolenic acid, see above.

The free fatty acids hexanoate and octanoate were fed at very low concentrations due to their toxic effects on plant growth. For hexanoate a large increase of the H6 monomer was observed, while octanoate resulted in a very high increase of the H8 monomer together with a moderate increase in the H6 monomer. For both substrates the H8:1 monomer content remained relatively high, which was probably due to the normal accumulation of PHA from endogenous lipid  $\beta$ -oxidation ("native" PHA).

Table 1. Increasing the total yield of PHA and changing its monomer composition in PHAC1 seedlings germinated in liquid media supplemented with fatty acids

Substrate	[sub] % (w/v)	light or dark (day 4 to 12)	fresh weight in mg <sup>b</sup>	mg PHA <sup>a</sup> per g fresh weight $\times 100$	% of total PHA (w/w)					
					H6	H8	H8:1	H10	H12	H14
None		light	232	1.9	1.3	43	29	10	9.7	6.8
None <sup>c</sup>		dark	186 $\pm$ 25	4.4	1.4	42	32	9.2	9.2	6.4
TWEEN-20	5	light	142	64	3.8	37	1.1	27	28	3.4
TWEEN-20 <sup>c</sup>	5	dark	57 $\pm$ 24	70	4.0	41	1.5	25	25	2.9
TWEEN-60 <sup>c</sup>	5	dark	125 $\pm$ 55	9.7	2.2	37	17	16	18	10
TWEEN-80 <sup>c</sup>	5	dark	141 $\pm$ 34	6.5	3.2	44	20	15	14	4.0
Hexanoate (C6) <sup>c</sup>	0.05	dark	70 $\pm$ 3	11	30	32	21	7.0	7.4	3.1
Octanoate (C8) <sup>c</sup>	0.005	dark	125 $\pm$ 44	16	5.2	73	13	3.7	3.7	1.5

<sup>a</sup> The transesterified plant material (of specified weight) was in a volume of 1 mL chloroform, of which 1  $\mu$ L was analyzed by GC.

<sup>b</sup> An average of 30 seedlings were grown per sample.

<sup>c</sup> Samples were done in duplicate and the results were averaged.

In experiment #2 (Tables 2 and 3) the seedlings were germinated for 8 days under continuous illumination, then the growth medium was replaced by the same medium containing 5% (v/v) TWEEN-80 together with various fatty acids, the purpose of the TWEEN-80 was to solubilize the water-insoluble fatty acids. The samples were placed back under constant illumination for another 6 days before being harvested and analysed. All samples were done in duplicate and each sample contained approximately 45 seeds which were germinated together in a large capped test-tube. Negative controls with pART27 plants were done for each substrate in the identical fashion. None of the novel PHA-monomer peaks were found in these negative controls.

Feeding of the saturated fatty acid tridecanoic acid (C13) and the branched fatty acid 8-methyl-nonanoic acid (8M-C9) resulted in the incorporation of a whole range of novel monomers. The identity of all these novel monomers was established by GC-MS. All of them had an uneven number of carbon atoms in their acyl chains and could be directly

traced to the original fatty acid supplied in the medium or intermediates of its degradation by  $\beta$ -oxidation. For tridecanoic acid, transgenic PHAC1 plants were found to contain a polymer having H13-, H11-, H9- and H7-3-hydroxy-alkanoic acid monomers. In the case of 8M-C9 the two novel monomers, 8-methyl-3-D-hydroxy-nonanoic acid (8M-H9) and 6-methyl-3-D-hydroxy-heptanoic acid (6M-H7), retained the branched structure of the original substrate. This shows that the PHA synthase C1 was able to incorporate a large variety of monomers into the polymer, provided that intermediates having the proper conformation were generated. The descending order in terms of quantities of the novel monomers (H13>H11>H9>H7; and 8M-H9>6M-H7) suggests that the  $\beta$ -oxidation of these unusual fatty acids proceeds slowly, thus permitting more time for intermediate-sequestration by the PHA synthase. It is possible that the 3-hydroxy-acyl-CoA dehydrogenase (MFP) and some other enzymes of the  $\beta$ -oxidation cycle have a low substrate specificity for these fatty acids and their derived intermediates.

Feeding of petroselinic acid (C18:1, 6-cis) resulted in a large increase in the content of the H14 monomer. This observation was in agreement with the proposed scheme of its degradation by  $\beta$ -oxidation (Gerhardt, B., *Lipid metabolism in plants* (Moore, T. S., Jr., ed.), CRC Press Inc., pp. 527-565 (1993)). All unsaturated bonds in the cis-conformation starting at an even-numbered carbon in the acyl chain were proposed to present obstacles to the normal cycle of the  $\beta$ -oxidation and had to be circumvented by modifications of the pathway. This is because the D-3-hydroxy-acyl-CoA can be formed by the action of the enoyl-CoA hydratase (MFP) from 2-cis-enoyl-CoA (cis-unsaturated bond in even-numbered position), but the D-3-hydroxy-acyl-CoA cannot be utilized by the 3-hydroxy-acyl-CoA dehydrogenase (MFP), which can only act on the L-3-hydroxy-acyl-CoA. Three possible modifications were put forward: 1) An epimerase converts the D-3-hydroxy-acyl-CoA to the L-form. 2) A dehydratase (also called D-3-hydroxyacyl-CoA hydrolyase or D-specific 2-trans-enoyl-CoA hydratase II, see Engeland, K. and Kindl, H., *Eur. J. Biochem.* 200: 171-178 (1991)) converts the D-3-hydroxy-acyl-CoA to 2-trans-enoyl-CoA, which can then be reconverted to L-3-hydroxy-acyl-CoA by the enoyl-CoA hydratase I. 3) A 2,4-dienoyl-CoA reductase reduces the 2-trans-4-cis-acyl-CoA  $\beta$ -oxidation intermediate to the 3-cis-enoyl-CoA, which in turn will require the activity of an isomerase to form the 2-trans-enoyl-

CoA  $\beta$ -oxidation intermediate. The first two options would result in the generation of D-3-hydroxy-acyl-CoA intermediates which would be directly available to the PHA synthase. Thus the observation of the specific increase in the H14 monomer upon feeding with petroselenic acid fits well with the predicted modifications of the  $\beta$ -oxidation to bypass the cis-unsaturated bond at carbon 6 of petroselenic acid. The same modifications have also been used above to explain the presence of the 3-hydroxy-octenoyl monomer (H8:1) in the native PHA. It was speculated that this monomer was due to the degradation of 18:3, all-cis- $\Delta$ 9, 12, 15 and 16:3, all-cis- $\Delta$ 7, 10, 13 by  $\beta$ -oxidation. The high proportion of H8 monomer could similarly be due to the degradation of linoleic acid (18:2, all-cis- $\Delta$ 9,12) which is an abundant fatty acid in plant material.

The degradation of fatty acids containing hydroxy groups on even-numbered carbon atoms in either the D- or the L-conformation also poses obstacles to the normal  $\beta$ -oxidation pathway and modifications are required to bypass these (Gerhardt, B., *Lipid metabolism in plants* (Moore, T. S., Jr., ed.), CRC Press Inc., pp. 527-565 (1993)). The D-4-hydroxy-decanoate-CoA and D-2-hydroxy-octanoate-CoA intermediates were predicted to arise in the degradation of ricinoleic acid (D-12-hydroxy-oleic acid (9-cis)). To investigate whether these intermediates might be incorporated into the PHA polymer by the PHA synthase, ricinoleic acid was used to supplement the medium in which PHAC1 plants were germinating. No major peaks due to the incorporation of novel monomers into the PHA polymer were detected, but GC-MS analysis was utilized to search for specific predicted novel monomers by looking for characteristic fragmentation products, namely ions 117 and 89. A small peak was found with ion 117, this peak showed the fragmentation fingerprint of the D-4-hydroxy-decanoate-methyl ester and was absent in the corresponding negative control. No novel peak was found with ion 89, thus excluding the possibility that the D-2-hydroxy-octanoate was incorporated into the polymer. It is known that the PHA synthase can incorporate D-4-hydroxy- and D5-hydroxy monomers into PHA in bacterial cultures, therefore the incorporation of the D-4-hydroxy-decanoate in the germinating seeds fed with ricinoleic acid was plausible. The very low abundance of the monomer could perhaps be explained by an alternative and more efficient pathway for the degradation of ricinolate



(Gerhardt, B., *Lipid metabolism in plants* (Moore, T. S., Jr., ed.), CRC Press Inc., pp. 527-565 (1993)).

Table 2. Quantity of PHA production in PHAC1 seedlings germinated in liquid medium supplemented with fatty acids

Substrate	[Substrate] % (w/v)	fresh weight (mg)	mg PHA <sup>a</sup> per gram fresh weight x 0.01
None		458 ± 8	4.6
5% TWEEN-80 (T)		549 ± 43	5.0
Tridecanoic acid (C13) + T	0.1	276 ± 9	28
8-methyl-nonanoic acid (8M-C9) + T	0.1	48 ± 14	46
Petroselenic acid (C18:1, 6-cis) + T	1	287	9.4
Ricinoleic acid (D12-OH-C18:1, 9- cis) + T	0.1	215 ± 21	6.0

<sup>a</sup> The plant material (of specified weight) was transesterified in different volumes, but the integrated peak-areas were calculated to homologate the sample-volumes (1 mL chloroform, of which 1 µL was analyzed by GC).

Table 3. Monomer composition of PHA produced in PHAC1 seedlings germinated in liquid medium supplemented with fatty acids

Substrate	% of total PHA (w/w)												
	H6	H7	6M-H7 <sup>a</sup>	H8	H8:1	H9	8M-H9 <sup>a</sup>	H10	4OH-H10 <sup>b</sup>	H11	H12	H13	H14
None	1.0			36	28			10			14		10
5% TWEEN 80 (T)	2.1			44	14			19			15		6.3
Tridecanoic acid (C13) + T	0.46	9.6		9.1	2.4	18		3.4		20	3.2	32	1.5
8-methyl-nonanoic acid (8M-C9) + T	0.20		24	9.2	3.0		55	3.0			2.8		2.2
Petroselinic acid (C18:1 6-cis) + T	1.6			32	7.4			17			17		26
Ricinoleic acid (D12 OH-C18:1, 9 cis) + T	1.8			46	8.8			30	1.2 <sup>c</sup>		7.4		5.0

<sup>a</sup> 8M-H9 and 6M-H7 refer to 8-methyl-3-D-hydroxy-nonanoic acid and 6-methyl-3-D-hydroxy-heptanoic acid, respectively.

<sup>b</sup> 4-OH-H10 refers to D-4-hydroxy-decanoate.

<sup>c</sup> The quantity of 4-OH-H10 was estimated by comparing peak sizes with H6 on a GC-MS chromatogram.

#### EXAMPLE 8: Extraction of high molecular weight PHA

The presence of derivatized monomers of PHA in PHAC1 plants had been established by the GC-analysis of trans-esterified intact plant material. To prove that the PHA was synthesized as high-molecular weight polymer and for its physico-chemical characterization, the purification of large quantities (i.e. in the mg range) was undertaken. Seeds of PHAC1#3.3 were germinated in liquid medium with and without addition of TWEEN-20 in order to obtain TWEEN-20-derived PHA or unmodified PHA, respectively.

For the TWEEN-20-derived PHA, approximately 16000 seeds (313 mg dry seeds) were germinated in 900 mL 1/2xMS + 1% sucrose medium for 7 days under continuous illumination on a shaker, the medium was replaced with 1/2xMS + 2% sucrose containing 5% TWEEN-20 and the seedlings were grown for another 9 days in the light. The plant material was harvested, washed extensively with water to remove residual TWEEN-20, frozen and lyophilized. The dry material was ground with a mortar and pestle, weighed, and lipids were extracted by a six-hour Soxhlet-extraction with methanol. The methanol-insoluble PHA was extracted for 24 hours in the same manner with chloroform. The chloroform extract was concentrated under reduced pressure and the PHA was precipitated by the addition of 10 volumes of cold methanol. This methanol precipitation was performed twice to ensure a high purity of the PHA. 27 mg of PHA was thus obtained from 5.35 g lyophilized and powdered seedling material, which related to 0.50% weight/dry weight. The PHA was trans-esterified and analyzed by GC. It was found that 58% of the PHA present in the methanol-extracted plant powder was extracted by the chloroform. It has been established in previous experiments that this remaining PHA was recalcitrant to extraction. The chromatogram showed that the extracted PHA was adequately pure with the peaks of the six identified monomers constituting 93% of the total integrated area. The ratio of the integrated areas between the different monomers was very similar to the result shown in Table 1 for the sample containing TWEEN-20 and grown under light, see Table 4.

For the extraction of high-M<sub>r</sub> PHA produced by PHAC1 plants without additional fatty acid supplements (native PHA), 1076 mg seeds (approx. 54000 seeds) were germinated in 3.3 L liquid medium (1/2xMS, 2% sucrose). The seeds were germinated under continuous illumination for 6 days, thereafter the medium was replaced and the seedlings put into the dark for another 7 days in order to induce plant senescence. The PHA was extracted from the plant material as above and one methanol precipitation was performed to purify the PHA. 23 mg of PHA was obtained from 14.3 g dry plant material, which related to 0.16 % weight/dry weight. It was determined that ≥69 % of the PHA had remained in the plant material after the chloroform extraction, which could be due to either the high content of C8:1 monomer (see Table 5) causing the polymer to "stick", or due to moisture in the ground material which had not been lyophilized completely, or due to the

large sample size for which a longer and more efficient chloroform extraction might have been required. The purification of native PHA and analysis by GC-MS allowed the detection of several more peaks that could not be initially resolved in crude extracts because of the high level of noise in the chromatogram. A total of eighteen 3-hydroxyacid monomers could be detected in the polymer (Table 1). In addition to 3-hydroxyhexanoic acid (H:6), 3-hydroxyoctanoic acid (H:8), 3-hydroxydecanoic acid (H:10), 3-hydroxydodecanoic acid (H:12), 3-hydroxytetradecanoic acid (H:14) and 3-hydroxyoctenoic acid (H8:1) monomers previously detected in the transesterification of intact plant material (crude extract) (Table 1), novel saturated and unsaturated monomers were detected which include 3-hydroxyhexadecanoic acid (H:16), 3-hydroxynonanoic acid (H9), 3-hydroxyundecanoic acid (H:11), 3-hydroxytridecanoic acid (H:13), 3-hydroxyhexadecatrienoic acid (H16:3), 3-hydroxyhexadecadienoic acid (H16:2), 3-hydroxyhexadecenoic acid (H16:1), 3-hydroxytetradecatrienoic acid (H14:3), 3-hydroxytetradecadienoic acid (H14:2), 3-hydroxytetradecenoic acid (H14:1), 3-hydroxydodecadienoic acid (H12:2) and 3-hydroxydodecenoic acid (H12:1). All even-chained monomers could be quantified and results are shown in Table 5.

It is expected that many of the unidentified minor peaks detected in the PHA purified from the TWEEN-20-fed seedlings would correspond to some of the minor saturated and unsaturated monomer detected in the "native" PHA.

Table 4. Comparison of the monomer composition of purified high-molecular weight PHA from Tween-20 feed plants with results obtained for transesterified intact seedlings during the preliminary feeding studies

Sample		% of total PHA area <sup>a</sup>					
		H6	H8	H8:1	H10	H12	H14
Purified high Mr PHA	TWEEN-20 derived	1.9	33.5	0.50	29	32	2.7
TWEEN-20 + light	see Table 1, line 3	3.8	37	1.1	27	28	3.

<sup>a</sup> Integrated area on the chromatogram.

Table 5. Monomer composition of "native" PHA isolated from *phaC1*-transformed plant line 3.3 grown in liquid media<sup>a</sup>

Monomer	H6	H8	H8:1	H10	H12	H12:1	H12:2
% (w/w)	1.1	23	18	4.7	5.8	4.3	5.0
std. dev.	0.16	4.4	4.6	0.51	0.46	0.60	1.3
Monomer	H14	H14:1	H14:2	H14:3	H16	H16:2	H16:3
% (w/w)	4.2	6.7	7.5	11	2.0	2.0	5.6
std. dev.	1.1	2.3	1.4	3.2	0.26	0.41	1.3

<sup>a</sup> Quantification of methyl esters was performed with a GC with a FID detector. Values were obtained from four separate PHA preparations. Monomers present in trace amounts (H9, H:11, H:13, H16:1) were not quantified.

#### EXAMPLE 9: Chemical characterization of high-molecular weight plant PHA

Purified TWEEN-20-derived PHA (13 mg) and unmodified PHA (5 mg) were given to Géraldine Coullerez at the EPFL (collaboration IBPV-EPFL) for the physico-chemical characterization of the polymer. Two different samples of bacterial PHA, PHA1 and PHOE, were obtained from Witholt and Kellerhals (ETH Zürich) to be used as controls. PHA1 contained predominantly H6 and H8 monomers (10% and 90%, respectively), while PHOE contained 4-10% H8:1, the balance being H6 and H8. The molecular weights and the respective dispersion coefficients of the polymers were determined by gel permeation chromatography (see Table 6). Polystyrene polymers were used as molecular weight standards. The results clearly show that the TWEEN-20 derived PHA produced by the transgenic plants is in the form of a high-M<sub>r</sub> polymer (about 200-250 monomers), although the molecular weight is only 20-25% of the bacterial polymers (about 1000 monomers). This shorter polymer length can be explained by an overabundance of PHA synthase relative to its substrate concentration and similar results have also been obtained in *in vitro* polymerization assays with purified PHB synthase (Jun Sim, S. et al., *Nature Biotechnology* 15: 63-67 (1997)). It is also possible that PHA polymers with longer chain lengths are trapped in the plant material, since a significant proportion of the PHA seems to be

recalcitrant to chloroform extraction ( $\geq 50\%$ , difficult to determine exact amounts in the trans-esterification of intact or powderized plant material, see above).

NMR analysis of the plant and bacterial PHAs revealed, that the TWEEN-20 derived plant PHA had the same structure as the bacterial PHA. The NMR spectrum of the unmodified plant PHA showed the peaks characteristic for the PHA polymer backbone, as well as several other peaks which have not been properly assigned or identified at this stage, but which could be due to various unsaturated bonds in the side chains of the polymer.

Table 6. Comparison of molecular weights of high-Mr PHA<sub>mcl</sub> purified from plants and bacteria

Origin of PHA	Mw	Mn	Dispersion Index
TWEEN-20 derived PHA - <i>Arabidopsis</i> PHAC1#3.3	$4.01 \times 10^4$	4910	8.17
PHA1 from <i>Pseudomonas oleovorans</i>	$1.46 \times 10^5$	58850	2.48
PHOE from <i>P. oleovorans</i>	$2.0 \times 10^5$	81590	2.44

**EXAMPLE 10:** The multifunctional protein (MFP) from the yeast *Candida tropicalis*

In animals, plants and bacteria,  $\beta$ -oxidation has been shown to proceed via the L-isomer of the 3-hydroxy-acyl-CoA intermediates and any D-isomers (which are predicted to arise in the degradation of fatty acids containing cis-unsaturated bonds at even-numbered carbons) have to be converted to the L-form in order to be oxidized further by the dehydrogenase activity of the multifunctional protein (MFP). In yeast the  $\beta$ -oxidation was reported to proceed via the D-isomer (Nuttley, W. M. et al., *Gene* 69: 171-180 (1988); Hiltunen, J. K. et al., *J. Biol. Chem.* 267: 6646-6653 (1992); Fosså, A. et al., *Mol. Gen. Genet.* 247: 95-104 (1995)). The yeast multifunctional protein (MFP) was shown to contain enoyl-CoA hydratase II and D-3-hydroxyacyl-CoA dehydrogenase activities, which together converted trans-2-enoyl-CoA via D-3-hydroxyacyl-CoA to 3-ketoacyl-CoA, i.e. the D-isomer was directly utilized by the dehydrogenase without prior conversion to the L-form.

It is anticipated that expression of this hydratase II activity together with the PHA synthase in the peroxisomes of double-transgenic plants will generate more of the D-3-hydroxy-acyl-CoA intermediates for their incorporation by the PHA synthase into the PHA polymer, thus increasing the final yield of PHA. Four separate approaches are envisioned.

5        A. Expression of the unchanged MFP from *C. tropicalis* in *A. thaliana*.

Since the hydratase II activity forms part of the MFP it was decided to perform investigatory experiments with the complete MFP prior to attempting to abolish the D-3-hydroxyacyl-CoA dehydrogenase activity. As the fungal MFP already had a peroxisomal  
10        targeting signal, this protein was expected also to be targeted to the plant peroxisomes.

The *C. tropicalis* MFP cDNA (Nuttley, W. M. et al., *Gene* 69: 171-180 (1988), GenBank Accession Number M22765) was cloned via PCR amplification (SEQ ID NO:21, encoding SEQ ID NO:22) into pART7 to obtain pART7\_MFP. The NotI-cassette, containing the CAMV35S-promoter in front of the MFP gene and the ocs3'- terminator, was  
15        inserted into the plant binary vector pART27 to obtain pART27\_MFP, which was transformed into *Arabidopsis*. Transgenic plant were selected on kanamycin and screened for the expression of the MFP protein with an anti-MFP antiserum. Homozygous T2 plants were cross-fertilized with PHAC1#3, PHAC1#4 and PHAC1#9 plants. Offspring from these crosses will be analyzed for their ability to biosynthesize PHA.

20        B. Changing the peroxisomal targeting signal of the yeast multifunctional protein (MFP) from -AKI to -SKL.

The COOH-terminal tripeptide -AKI was shown to be responsible for peroxisomal targeting of the MFP in yeast, but it has not yet been demonstrated to function in plant  
25        peroxisomal targeting. The MFP.SK gene, in which the 3'-terminal nucleotide sequence of the MFP gene encoding the -AKI tripeptide had been changed to -SKL by PCR site-directed mutagenesis (SEQ ID NO:23, encoding SEQ ID NO:24), was obtained from the laboratory of K. Hiltunen to ascertain that the MFP was properly targeted to the plant peroxisomes and

to serve as a positive control in targeting studies with the yeast multifunctional protein (MFP) in plant cells. The MFP.SKL gene was used to construct pART7\_MFP.SKL. The NotI-cassette of pART7\_MFP.SKL, containing the MFP-SKL gene flanked by the CaMV35S promoter and the ocs3'-terminator, was cloned into pART27 to obtain pART27\_MFP.SKL, which was transformed into *A. thaliana* ecotype Columbia. Kanamycin resistant T1 plants were obtained. The high-MFP.SKL-expressing lines will be selected by Western analysis of T2 plants, and the selected lines will be crossed with PHAC1#3.3 plants.

C. Deleting the peroxisomal targeting signal of the yeast multifunctional protein (MFP).

The construct pART7\_MFPΔAKI was obtained by PCR amplification of the MFP gene such that the 3'-terminal nucleotide sequence of the MFP gene encoding the -AKI tripeptide was deleted by the introduction of a stop codon (SEQ ID NO:25, encoding SEQ ID NO:26). The "detargeted" MFPΔAKI is expected to be localized in the cytoplasm and will be utilized as negative control in experiments to study the localization of MFP and MFP.SKL in plant cells. pART27\_MFPΔAKI was transformed into *A. thaliana* ecotype Columbia and Kanamycin resistant T1 plants were obtained. The high-MFPΔAKI-expressing lines will be selected by Western analysis of T2 plants and these lines will be crossed with PHAC1#3.3 plants.

D. Deleting the dehydrogenase activity of the yeast multifunctional protein (MFP).

As only the hydratase II activity of the yeast multifunctional protein (MFP) is of interest, plants will be transformed with the MFPADH gene, in which the dehydrogenase activity was deleted by site-directed mutagenesis of specific amino acid residues identified as being essential for this activity.

EXAMPLE 11: Verification of enzyme activity of modified MFP constructs in *Pichia*



The modified MFP.SKL and MFP $\Delta$ AKI genes were subcloned from pART7\_MFP.SKL and pART7\_MFP $\Delta$ AKI into the yeast expression vector pHILD2. The resulting plasmids pHILD2\_MFP.SKL and pHILD2\_MFP $\Delta$ AKI were transformed into *Pichia* and enzyme assays were performed in Hiltunen's laboratory. Results indicated that the modifications to the genes did not have an effect on the dehydrogenase and the hydratase enzymatic activities.

EXAMPLE 12: Expression of the FatB3 acyl-ACP thioesterase in double transgenics to increase PHA yield

Expresion of the California bay acyl-ACP thioesterase was shown to cause premature termination of fatty acid elongation during fatty acid biosynthesis in transgenic oilseed plants (Voelker, T. A. et al., *Science* 257: 72-74 (1992)). The resulting medium-chain-length fatty acids were found to accumulate in the triglycerides of seed lipids, but could not be detected in leaves. It is thought that medium chain fatty acids do not accumulate in the leaves of transgenic plants because they get degraded immediately by  $\beta$ -oxidation (Eccleston, V. S. et al., *Planta* 198: 46-53 (1996)). This increased flux of medium-chain fatty acids through  $\beta$ -oxidation may be exploited to improve the yield of PHA, as well as to modify the composition of the polymer towards saturated H6-H14 monomers in double transgenic plants expressing both acyl-ACP thioesterase and the PHAC1 synthase.

The plasmid pBJ49\_FatB3 containing the *Cuphea lancolata* thioesterase FatB3 gene under control of a 200 bp minimal promoter derived from the 35S promoter was infiltrated into the *A. thaliana* PHAC1#3.3 transgenic line which is homozygous for the PHAC1 gene. Hygromycin resistant lines were obtained and the seed lipid content of T1 seeds was analysed for increased levels of medium chain length fatty acids and 11 separate lines expressing high levels of the acyl-ACP thioesterase were identified in this manner. Subsequently the polyhydroxyalkanoate content of leaves from soil grown T2 double transgenic offspring was determined by GC and GC-MS analysis of the 3-hydroxy-fatty

acid methyl esters obtained by transesterification of whole leaves. The results (Table 7) indicated an approximate tenfold increase in the polyhydroxyalkanoate content of leaves from double transgenic plants when compared to plants expressing only the PHAC1 synthase. The increased polyhydroxyalkanoate yield was mainly due to a large increase in the content of the saturated polyhydroxyalkanoate monomers with an even number of carbons, namely 3-OH-octanoate (H8), 3-OH-decanoate (H10), 3-OH-dodecanoate (H12) and 3-OH-tetradecanoate (H14) (Table 8).

The recombinant FatB3 acyl-ACP thioesterase is naturally targeted to the chloroplast, where it removes medium chain-length acyl-ACP intermediates from the fatty acid biosynthesis. These short chain fatty acids accumulate in the seed lipids, but not in the leaves of transgenic plants and it has been speculated, that they are immediately degraded by  $\beta$ -oxidation. Results with these double transgenic plants indicate that there is indeed an increase in the  $\beta$ -oxidation of medium chain length fatty acids in the leaves, which results in a higher yield of polyhydroxyalkanoate due to the incorporation of the  $\beta$ -oxidation intermediates into the PHA by the polyhydroxyalkanoate synthase.

Table 7. PHA content of leaves from single and double transgenic plants expressing the PHAC1 synthase alone or together with the FatB3 acyl-ACP thioesterase

Plants	PHA content (mg/g fresh weight)		
		average	std. deviation
PHAC1#3.3 plant 1	0.0040		
PHAC1#3.3 plant 2	0.0253	0.0147	0.015
PHAC1#3.3 + FatB3 line 2.4a plant 2	0.1281		
PHAC1#3.3 + FatB3 line 2.4b plant 1	0.0749	0.1175	0.038
PHAC1#3.3 + FatB3 line 2.4b plant 5	0.1495		

Table 8. PHA content of leaves from single and double transgenic plants expressing the PHAC1 synthase alone or together with the FatB3 acyl-ACP thioesterase

Monomer	PHAC1#3.3			PHAC1#3.3 + FatB3		
	mg/g	std. dev.	% (w/w)	mg/g	std. dev.	% (w/w)
H6	0.00035	0.00036	2.39	0.00455	0.00131	3.87
H8:1	0.00451	0.00525	30.76	0.00790	0.00913	6.73
H8	0.00205	0.00201	13.94	0.03765	0.01120	32.05
H9	0.00014	0.00001	0.95	0.00029	0.00010	0.25
H10	0.00087	0.00080	5.91	0.04694	0.01816	39.96
H11	0.00017	0.00002	1.17	0.00034	0.00015	0.29
H12	0.00145	0.00145	9.87	0.00642	0.00247	5.47
H13	0.00016	0.00010	1.09	0.00023	0.00013	0.20
H14:1	0.00072	0.00059	4.92	0.00141	0.00114	1.20
H14:2	0.00121	0.00142	8.21	0.00179	0.00209	1.53
H14:3	0.00086	0.00106	5.86	0.00142	0.00178	1.20
H14	0.00219	0.00222	14.93	0.00853	0.00459	7.26

**EXAMPLE 13: Crossing PHAC1#3.3 transgenic plants with fatty acyl hydroxylase**

**LFah12 transgenic plants**

Three lines of transgenic *A. thaliana* expressing the LFah12 fatty acyl hydroxylase gene from *Lesquerella* were obtained from Pierre Broun (Chris Somerville's laboratory, Carnegie Institution, Stanford, CA). This fatty acyl hydroxylase is responsible for the production of ricinoleic acid (C18:1; 9-cis, D-12-hydroxy) in *Lesquerella*. It was found that hydroxylated fatty acids accumulated in the seed triglycerides of *Arabidopsis*, but not in the leaves, again indicating that hydroxylated fatty acids synthesized in leaves are most likely degraded by  $\beta$ -oxidation (Broun, P. and Somerville, C., *Plant Physiol.* 113: 933-942 (1997); van de Loo, F.N. et al., *Proc. Natl. Acad. Sci. U.S.A.* 92: 6743-6747 (1995)). Crosses were made with the three fatty acyl hydroxylase transgenic lines and the PHAC1#3.3 line and the seeds of these crosses were harvested. Seeds and their progeny plants will be examined for their levels of PHA biosynthesis. The aim of this experiment is to investigate if the increased flux of hydroxylated fatty acids to the  $\beta$ -oxidation cycle in transgenic plants expressing the Fah 12 and PHA synthase genes can lead to an increase in the yield of PHA and if novel hydroxylated monomers can be incorporated in the PHA.

**EXAMPLE 14: Influence of carbon source and light conditions on PHA synthesis**

The amount of PHA present in plant tissues was influenced by the growth conditions. For plants grown for three weeks under constant illumination in MS liquid media with 2% sucrose, the yield of PHA was approximately 0.6 mg/g dry weight (dwt). Removal of sucrose for the last week of growth in the light resulted in a 100% increase in PHA, while plants growing in 2% sucrose but shifted in the dark for the last week accumulated 22% more PHA (Table 9).

Table 9. Influence of sucrose and light on PHA accumulation in phaC1-transformed line 3.3

PHA yield	Growth conditions <sup>a</sup>					
	0% sucrose	0.2% sucrose	2% sucrose	0% sucrose	0.2% sucrose	2% sucrose
	dark	dark	dark	light	light	light
mg PHA/g dwt	1.42	1.31	0.73	1.23	1.08	0.60
Relative % <sup>b</sup>	100	92	52	87	76	42

<sup>a</sup> Seedlings were grown under constant illumination in a liquid medium containing MS salts and 2% (w/v) sucrose for 2 weeks, and then grown for another week, either in the dark or in the light, in media containing different concentrations of sucrose.

<sup>b</sup> The yield of 1.42 mg/g dry weight was arbitrarily defined as 100%.

**EXAMPLE 15: Peroxisome targeting**

It has been shown in multiple systems (e.g., yeast, animal, and plants) that targeting of proteins to the peroxisome can be achieved by the addition of as little as three amino acids at the carboxy end of a foreign protein (see Gietl, C., *Physiol. Plant.* 97: 599-608 (1996); Purdue, P.E. and Lazarow, P. B., *J. Biol. Chem.* 269: 30065-30068 (1994); Subramani, *Ann. Rev. Cell Biol.*, 9: 445-478 (1993)). The minimal consensus sequence for peroxisome targeting of protein via the carboxy end, named PTS1 for peroxisomal targeting sequence 1, is a small uncharged amino acid at position 1 (S, A, or P), a positively-charged

amino acids at position 2 (K, R, S, or H), and a hydrophobic amino acid at position 3 (L, M, I or F).

Thus, although the initial minimal PTS1 sequence was defined as SKL, a range of substitution have been found to be effective PTS1 signal, including ARM, SRM, SKL, ARL, SRL, PSI, or PRM. Specific examples of targeting of foreign proteins in plants include: 6 amino acid PTS1 (RAVARL, Volokita, M., *Plant J.* 1: 361-366 (1991)); 5 amino acids PTS1 (AKSRM, Olsen, L. J. et al, *Plant Cell* 5: 941-952 (1993)); 4 amino acids PTS1 (KSRM, Trelease, R. N. et al., *Protoplasma* 195: 156-167 (1996)); 5 amino acid PTS1 (ELSRL, Hayashi, M et al, *Plant J.* 10: 225-234 (1996)); 4 amino acid PST1 (RPSI, Mullen R. T. et al, *Plant J.* 12: 313-322 (1997)); 3 amino acid PTS1 (SKL, Banjoko, A. et al., *Plant Physiol* 107: 1201-1208 (1995)); 3 amino acid PTS1 (ARM, Lee, M.S. et al., *Plant Cell* 8: 185-197 (1997)).

A comparison of the peroxisomal targeting sequence 1 (PTS1) found in mammals, fungi and trypanosomes was performed by Purdue, P.E. and Lazarow, P.B. (*J. Biol. Chem.* 269: 30065-30068 (1994)). All sequences shown in Table 10 are functional in at least one species. Other sequences may or may not have been tested. For trypanosomes, all sequences with a single amino acid change from SKL that are not shown are nonfunctional. The asterisks refer to the fact that -NKL and -SQL (outside the mammalian consensus, but not directly tested) have been found at the C termini of mammalian peroxisomal proteins. Uppercase, functional; lowercase, nonfunctional; underlined, not yet found on a peroxisomal protein in that species.

Table 10. C-terminal peroxysomal targeting sequences.

Mammals	<i>S. cerevisiae</i>	<i>H. polymorpha</i>	<i>C. albicans</i>	Trypanosomes
SKL	SKL	<u>SKL</u>		<u>SKL</u>
SRL				SRL
SHL				SHL
AKL				AKL
<u>CKL</u>				<u>CKL</u>
<u>skf</u>	SKF			
<u>ski</u>		SKI		<u>SKI</u>
*		NKL		<u>NKL</u>
		ARF		
	<u>AKI</u>		<u>AKI</u>	
	<u>aqi</u>		<u>AOI</u>	
	<u>gki</u>		<u>GKI</u>	
<u>ssl</u>				SSL
				SKM
<u>tkl</u>				<u>G/H/P/T-KL</u>
*				<u>S-M/N/Q-L</u>
				<u>SKY</u>

The minimal peroxisomal targeting sequence 1 (PTS1) in plants has been found to be ARM, SRM, SKL, ARL, SRL, PSI, and PRM (Compilation from Volokita, M., *Plant J.*, 1: 361-366 (1991); Olsen, L.J. et al., *Plant Cell*, 5: 941-952 (1993); Trelease, R.N. et al., *Protoplasma*, 195: 156-167 (1996); Gietl, C., *Physiol. Plant.*, 97: 599-608 (1996); Purdue, P.E. and Lazarow, P.B., *J. Biol. Chem.*, 269: 30065-30068 (1994); Subramani, *Ann. Rev. Cell Biol.*, 9:445-478 (1993); Mullen, R.T., et al., *Plant J.*, 12: 313-322 (1997); Lee, M.S., et al., *Plant Cell*, 9: 185-197 (1997)).

Some proteins are targeted to the peroxisome via an N-terminal extension called  
 10 PTS2 for peroxisome targeting sequence 2. In this case, a consensus sequence of nine amino

acids has been defined, being (R/K)(L/Q/I)XXXXXX(H/Q)L. Foreign protein (eg  $\beta$ -glucuronidase) can also be targeted in plants to the peroxisome by adding a PTS2 sequence at the N-terminal end of the protein (Kato et al, *Plant Cell* 8: 1601-1611 (1996)).

**EXAMPLE 16: Co-expression of PHA with other sequences resulting in increased or novel PHA biosynthesis**

PHA<sub>mcl</sub> synthesized in transgenic plants can include a large variety of monomers, with functional groups that can be used to modify and improve the characteristics of the polymer before or after extraction from the plant. For example, the presence of double bonds, epoxy groups, or acetylated groups within the PHA may be used to cross-link the polymer. The examples herein have demonstrated the incorporation of the following range of monomers into plant PHAmcl: even-chain saturated 3-OH-acyl monomers with six to sixteen carbons; odd-chain saturated 3-OH-acyl monomers with seven to thirteen carbons; unsaturated 3-OH-acyl monomer with 8, 12, 14, and 16 carbons and with 1, 2, or 3 double bonds; branched-chain 3-OH-acyl monomers (8-methyl-3-D-hydroxy-nonanoic acid and 6-methyl-3-D-hydroxy-heptanoic acid) and 4-OH-acyl monomers (D-4-hydroxy-decanoate). Although in these experiments some monomers, such as branched-chain, odd-chain or hydroxylated 3-hydroxyacids, were found included in PHAs after exogenous fatty acids were supplied to the transgenic plants, the same range of monomers would also be included in plant PHA from fatty acids supplied from endogenous fatty acid synthesis. Thus, one can predict being able to synthesize PHA polymers in plants that have a wide range of monomers, for example, higher proportion of short-chain monomers, unsaturated bonds at novel positions, monomers with hydroxylated groups, epoxy groups, acetylated groups, keto groups, cyclopentenyl groups, cyclopropanoid groups, furanoid groups or halogenated groups, branched chain, cyclic groups or any other novel monomers for which the equivalent functional groups exist in fatty acids in plants. The incorporation of these novel monomers derived from fatty acids into plant PHAs could be accomplished by expressing a PHA synthase in a plant which synthesizes these unusual fatty acids either naturally or after expression of a transgene such as fatty-acyl-thioesterases, -hydroxylases, -desaturases, -epoxidases, or -acetylases.

It is also conceivable that the substrate specificity of the PHA synthase could be modified to allow the incorporation of a wider range of monomers into PHA. One can predict that the range of monomers which could be included into plant PHAs from such a modified PHA synthase will include monomers that can be derived from plant fatty acid metabolism found in wild type plants or plants expressing transgenes (such as desaturases, hydroxylases, thioesterases, epoxidases, acetylases) which results in the modification of fatty acids synthesized in plants. It is also conceivable that suitable hydroxy acid substrates for the PHA synthase can be obtained from the amino acid metabolism or the plant secondary metabolism.

It has been demonstrated before that plants can synthesize PHB from acetyl-CoA through the expression of the 3-ketothiolase, acetoacetyl-CoA reductase and PHB synthase from *A. eutrophus* (Poirier, Y. et al., *Science* 256: 520-523 (1992); Nawrath, C. et al., *Proc. Natl. Acad. Sci. U.S.A.* 91: 12760-12764 (1994)). The examples herein demonstrate that PHA<sub>mcl</sub> can be synthesized in plants expressing a PHA synthase which can accept monomers from H6-H16. Since acetyl-CoA is also found in the peroxisome, one can predict that co-expression of a PHA synthase with a substrate specificity for 3-hydroxyacids ranging from H4 to H8 or higher in the peroxisome, and of the *A. eutrophus* acetoacetyl-CoA reductase, would lead to the biosynthesis of a copolymer containing hydroxybutyrate and hydroxyacids of H6 and higher. In this pathway, the expression of the 3-ketothiolase from *A. eutrophus* may not be required since the peroxisome already contains a 3-ketothiolase.

The examples herein clearly show that synthesis of PHA in plants can be significantly enhanced by increasing the pool of fatty acids which is channeled through  $\beta$ -oxidation. Thus, when short-chain fatty acids were added externally in the form of TWEEN-20 to PHAC1-transgenic plants, there was a 30- fold increase in the amount of PHA synthesized in plants. Similar large increases in PHA synthesis were found when tridecanoic acid and 8-methyl-nonanoic acid were added to the growth media. It is hypothesized that because these fatty acids could not be incorporated into membranes without disrupting them, the fatty acids are detoxified by channeling them to the peroxisome



for degradation by the  $\beta$ -oxidation cycle. Thus, increased channeling of fatty acids to the  $\beta$ -oxidation cycle results in an increase in PHA synthesized using intermediates of fatty acid oxidation. One can predict from this work that any changes in plants which results in an increased flux of fatty acids to the  $\beta$ -oxidation cycle will results in an increase in PHA synthesis in plants expressing a PHA synthase targeted to the peroxisome. Increasing the flux of fatty acids to the  $\beta$ -oxidation cycle could be accomplished by overexpressing enzymes which lead to the biosynthesis of modified fatty acids. This has been demonstrated in plants expressing thioesterase (Eccleston, V.S. et al., *Planta* 198: 46-53 (1996)) and implied in plants expressing hydroxylase (van de Loo, F.N. et al., *Proc. Natl. Acad. Sci. U.S.A.* 92: 6743-6747 (1995)). Increase of flux of lipids to the  $\beta$ -oxidation cycle and to PHA synthesis could also be accomplished by expressing other fatty acid modifying enzymes, such as desaturases, epoxidases, acetylases, enzymes involved in synthesis of branched-chain fatty acids, etcetera. This concept has been directly demonstrated in this present work with a fatty acyl-ACP thioesterase. It was shown that co-expression of a fatty acyl-ACP thioesterase in a plant expressing a peroxisomal PHA synthase leads to a 10 fold increase in PHA (Table 7). In addition of increasing the amount of PHA in plants , expression of the thioesterase leads to a predictable change in the composition of the PHA, i.e. since the *C. lanceolata* FatB3 thioesterase has the highest affinity for saturated C10 fatty acyl-ACP, there is a corresponding large increase in hydroxydecanoic acid (H10) present in the plant PHA (Table 8). Thus, expression of fatty acid modifying enzymes in conjunction with a PHA synthase in plants not only leads to an increase in the amount of PHA synthesized in plants, but also leads to a predictable changes in the PHA monomer composition, e.g. co-expression of a short-chain fatty acyl-ACP thioesterase would lead to an increase in the proportion of short-chain hydroxyacid monomers in plant PHA, co-expression of a long-chain fatty acyl-ACP thioesterase would lead to an increase in the proportion of long-chain hydroxyacid monomers in plant PHA, co-expression of a fatty acyl hydroxylase would lead to an increase in the proportion of hydroxylated hydroxyacid monomers in plant PHA, co-expression of a fatty acyl epoxidase would lead to an increase in the proportion of epoxidated monomers in plant PHA, co-expression of a fatty acyl acetylase would lead to an increase in the proportion of acetylated hydroxyacid monomers in plant PHA, and co-expression of a fatty acyl desaturase would lead to an increase in the

proportion of unsaturated hydroxyacid monomers in plant PHA. Increase in flux of lipids through the  $\beta$ -oxidation cycle could also be accomplished by overexpressing the key regulators (i.e. transcriptional factors) involved in the up-regulation of the entire  $\beta$ -oxidation cycle pathway during germination or senescence. This last approach would have the advantage of turning-on the  $\beta$ -oxidation cycle in tissues which normally have only low activity, such as the developing seeds of oil crops.

The examples herein point out the impact of fatty acid modifying enzymes for the production of novel PHA in transgenic plants expressing a PHA synthase. One key enzyme appears to be a 3-hydroxy-acyl-CoA epimerase. Although the normal function of the epimerase is to convert D-3-hydroxy-acyl-CoAs to the L-form required for the action of the L-3-hydroxy-acyl-CoA dehydrogenase, the reverse reaction of the epimerase can be responsible for converting the L-form to the D-form, which is essential for the activity of the PHA synthase. For that purpose the epimerase is important for the supply of the substrates for the PHA synthase derived from  $\beta$ -oxidation in the peroxisomes. Recombinant forms of such an epimerase activity expressed in peroxisomes or in other plant cell compartments like the cytoplasm or the plastids could play an important role in the production of PHA in transgenic plants. It is possible that the slow rate of the epimerase "reverse reaction" could be the major factor limiting the supply of substrates for the PHA synthase. The substrate limitation due to this could be the reason why PHA synthesis seemed to have reached a maximum in seedlings germinated both in the light and in the dark in liquid medium supplemented with TWEEN-20, which contains only saturated fatty acids.

The importance of certain fatty acid desaturases is highlighted by Table 3, wherein petroselinic acid (C18:1, 6-cis) was supplied to germinating PHAC1#3.3 seedlings in liquid medium, resulting in the specific increase of the H14 monomer. This indicated that any fatty acid containing unsaturated bonds starting at even-numbered carbons directly gives rise to the appropriate D-3-hydroxy-acyl-CoAs during  $\beta$ -oxidation, thus bypassing the otherwise necessary "reverse reaction" of the epimerase to generate the D-intermediates. Similarly the H8 and the H8:1 monomer are predicted to originate from the unsaturated fatty

acids linoleic acid (C18:2, 9,12-all cis) and linolenic acid (C18:3, 9,12,15-all cis). For that reason any plant containing high levels of fatty acids with unsaturated bonds starting at even-numbered carbons could be of interest for the production of PHA<sub>met</sub>, or the transgenic expression of suitable fatty acid desaturases producing such unsaturated fatty acids in plants containing the PHA synthase would be similarly attractive for PHA production and monomer manipulation.

The examples herein demonstrate that a peroxisomally-located PHA synthase is able to divert intermediates from  $\beta$ -oxidation for their incorporation into PHA. The existence of the required D-3-hydroxy-acyl-CoA substrates was important for the synthesis of PHA. In light of the present disclosure, one may predict that PHA can be produced in a similar manner in any other compartment of any plant cell, provided that a supply of such D-3-hydroxy-acyl-CoA intermediates is present due either to an endogenous metabolic pathway or due to an artificially created pathway utilizing expression of transgenes. Fatty acid biosynthesis occurs in the plastids in plant cells, and modifications of this pathway could turn the plastids into a suitable source of D-3-hydroxy-acyl-CoA intermediates, which could subsequently be used to produce PHA either in the plastid itself or in other cell compartments.

#### EXAMPLE 17: Protein analysis

Leaves from transgenic plants were homogenized in 200 mM Tris-HCl (pH 7.5), 250 mM EDTA, 5 mM dithiothreitol and 1 mM phenylmethylsulfonyl fluoride. The homogenate was clarified by centrifugation and protein analyzed by Western blot using the ECL detection system (Amersham, Arlington Heights, IL).

#### EXAMPLE 18: Immunolocalization

Transgenic plants were grown on media containing MS salts, 1% sucrose, 0.7% agar and 50  $\mu$ g/mL kanamycin for either 7 days in the light or 1 day in the light followed by 6 days in the dark. Whole plants were fixed for 2 hours at room temperature in 4%

formaldehyde, 0.5% glutaraldehyde, 50 mM sodium cacodylate pH 7.3. The tissue samples were dehydrated in an ethanol series and embedded in LR White resin. Ultra thin sections were cut using a microtome, mounted on formvar-coated gold grids and blocked in 0.8% (w/v) bovine serum albumin, 0.1% (w/v) gelatine, 5% (w/v) normal goat serum and 2 mM sodium azide in PBS (10 mM sodium phosphate, 150 mM sodium chloride, pH 7.4). Grids were incubated for 1 hour at room temperature with antiserum against PHA synthase (1:50), glycolate oxidase (1:2000) and isocitrate lyase (1:1000) in the blocking solution followed by a 4 hour incubation at room temperature with a 1:50 dilution of gold-conjugated goat anti-rabbit antibodies (15 nm gold particles) in PBS. Immunolabeled sections were double-stained with uranyl acetate and lead citrate and viewed with a Jeol JEM transmission electron microscope.

#### EXAMPLE 19: PHA extraction and analysis

Fresh or dried frozen plant material was ground in a mortar and lyophilized. The powder was extracted with methanol in a Soxhlet apparatus for 24 hours followed by PHA extraction with chloroform for 24 hours, both at 85°C. The PHA-containing chloroform was concentrated under reduced pressure and extracted once with water to remove residual solid particles. PHA was precipitated by the addition of 10 volumes of cold methanol and subsequently washed by two cycles of chloroform solubilisation and methanol precipitation. PHA dissolved in chloroform was transesterified by acid methanolysis (Huijberts, G. N. et al., *Appl. Environ. Microbiol.* 58: 536-544 (1992)) and analyzed by gas-chromatography and mass spectrometry (GC-MS) using a Hewlett-Packard 5890 gas chromatograph (30 m long HP-5MS column) coupled to a Hewlett-Packard 5972 mass spectrometer (Hewlett Packard, Palo Alto, CA). Molecular weight determination of PHA samples were determined by gel permeation chromatography on a Waters 150 CV (Waters Corp., Milford, MA) equipped with a differential refractive index detector and an on-line viscometer and three ultrastyrigel columns in series ( $10^4$ ,  $10^5$  and  $10^6$  Å). Samples were prepared in dichloromethane and calibration performed using polystyrene standards.

EXAMPLE 20: Plant Vectors

In plants, transformation vectors capable of introducing encoding DNAs involved in PHA biosynthesis are easily designed, and generally contain one or more DNA coding sequences of interest under the transcriptional control of 5' and 3' regulatory sequences. Such vectors generally comprise, operatively linked in sequence in the 5' to 3' direction, a promoter sequence that directs the transcription of a downstream heterologous structural DNA in a plant; optionally, a 5' non-translated leader sequence; a nucleotide sequence that encodes a protein of interest; and a 3' non-translated region that encodes a polyadenylation signal which functions in plant cells to cause the termination of transcription and the addition of polyadenylate nucleotides to the 3' end of the mRNA encoding said protein. Plant transformation vectors also generally contain a selectable marker. Typical 5'-3' regulatory sequences include a transcription initiation start site, a ribosome binding site, an RNA processing signal, a transcription termination site, and/or a polyadenylation signal. Vectors for plant transformation have been reviewed in Rodriguez et al. (Vectors: A Survey of Molecular Cloning Vectors and Their Uses, Butterworths, Boston. (1988)), Glick et al. (Methods in Plant Molecular Biology and Biotechnology, CRC Press, Boca Raton, Fla. (1993)), and Croy (Plant Molecular Biology Labfax, Hames and Rickwood (Eds.), BIOS Scientific Publishers Limited, Oxford, UK. (1993)).

EXAMPLE 21: Plant Promoters

Plant promoter sequences can be constitutive or inducible, environmentally- or developmentally-regulated, or cell- or tissue-specific. Often-used constitutive promoters include the CaMV 35S promoter (Odell et al., *Nature* 313: 810 (1985)), the enhanced CaMV 35S promoter, the Figwort Mosaic Virus (FMV) promoter (Richins et al., *Nucleic Acids Res.* 20: 8451 (1987)), the mannopine synthase (*mas*) promoter, the nopaline synthase (*nos*) promoter, and the octopine synthase (*ocs*) promoter. Useful inducible promoters include promoters induced by salicylic acid or polyacrylic acids (PR-1, Williams, S. W. et al., *Biotechnology* 10: 540-543 (1992)), induced by application of safeners (substituted benzenesulfonamide herbicides, Hershey, H.P. and Stoner, T.D., *Plant Mol. Biol.* 17: 679-

690 (1991)), heat-shock promoters (Ou-Lee et al., *Proc. Natl. Acad. Sci U.S.A.* 83: 6815 (1986); Ainley et al., *Plant Mol. Biol.* 14: 949 (1990)), a nitrate-inducible promoter derived from the spinach nitrite reductase gene (Back et al., *Plant Mol. Biol.* 17: 9 (1991)), hormone-inducible promoters (Yamaguchi-Shinozaki et al., *Plant Mol. Biol.* 15: 905 (1990); Kares et al., *Plant Mol. Biol.* 15: 905 (1990)), and light-inducible promoters associated with the small subunit of RuBP carboxylase and LHCP gene families (Kuhlemeier et al., *Plant Cell* 1: 471 (1989); Feinbaum et al., *Mol. Gen. Genet.* 226: 449 (1991); Weisshaar et al., *EMBO J.* 10: 1777 (1991); Lam and Chua, *J. Biol. Chem.* 266: 17131 (1990); Castresana et al., *EMBO J.* 7: 1929 (1988); Schulze-Lefert et al., *EMBO J.* 8: 651 (1989)). Examples of useful tissue-specific, developmentally-regulated promoters include the  $\beta$ -conglycinin 7S promoter (Doyle et al., *J. Biol. Chem.* 261: 9228 (1986); Slighton and Beachy, *Planta* 172: 356 (1987)), and seed-specific promoters (Knutzon et al., *Proc. Natl. Acad. Sci U.S.A.* 89: 2624 (1992); Bustos et al., *EMBO J.* 10: 1469 (1991); Lam and Chua, *Science* 248: 471 (1991); Stayton et al., *Aust. J. Plant. Physiol.* 18: 507 (1991)). Plant functional promoters useful for preferential expression in seed plastids include those from plant storage protein genes and from genes involved in fatty acid biosynthesis in oilseeds. Examples of such promoters include the 5' regulatory regions from such genes as napin (Kridl et al., *Seed Sci. Res.* 1: 209 (1991)), phaseolin, zein, soybean trypsin inhibitor, ACP, stearyl-ACP desaturase, and oleosin. Seed-specific gene regulation is discussed in EP 0 255 378. Promoter hybrids can also be constructed to enhance transcriptional activity (Comai, L. and Moran, P.M., U.S. Patent No. 5,106,739, issued April 21, 1992), or to combine desired transcriptional activity and tissue specificity.

#### EXAMPLE 22: Plant transformation and regeneration

A variety of different methods can be employed to introduce such vectors into plant protoplasts, cells, callus tissue, leaf discs, meristems, etcetera, to generate transgenic plants, including *Agrobacterium*-mediated transformation, particle gun delivery, microinjection, electroporation, polyethylene glycol-mediated protoplast transformation, liposome-mediated transformation, etc. (reviewed in Potrykus, *Ann. Rev. Plant Physiol. Plant Mol. Biol.* 42: 205 (1991)). In general, transgenic plants comprising cells containing and expressing

DNAs encoding enzymes facilitating PHA biosynthesis can be produced by transforming plant cells with a DNA construct as described above via any of the foregoing methods; selecting plant cells that have been transformed on a selective medium; regenerating plant cells that have been transformed to produce differentiated plants; and selecting a transformed plant which expresses the enzyme-encoding nucleotide sequence.

Specific methods for transforming a wide variety of dicots and obtaining transgenic plants are well documented in the literature (Gasser and Fraley, *Science* 244: 1293 (1989); Fisk and Dandekar, *Scientia Horticulturae* 55: 5 (1993); Christou, *Agro Food Industry Hi Tech*, p.17 (1994); and the references cited therein).

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Successful transformation and plant regeneration have been reported in the monocots as follows: asparagus (*Asparagus officinalis*; Bytebier et al., *Proc. Natl. Acad. Sci. U.S.A.* 84: 5345 (1987)); barley (*Hordeum vulgare*; Wan and Lemaux, *Plant Physiol.* 104: 37 (1994)); maize (*Zea mays*; Rhodes et al., *Science* 240: 204 (1988); Gordon-Kamm et al., *Plant Cell* 2: 603 (1990); Fromm et al., *Bio/Technology* 8: 833 (1990); Koziel et al., *Bio/Technology* 11: 194 (1993)); oats (*Avena sativa*; Somers et al., *Bio/Technology* 10: 1589 (1992)); orchardgrass (*Dactylis glomerata*; Horn et al., *Plant Cell Rep.* 7: 469 (1988)); rice (*Oryza sativa*, including indica and japonica varieties; Toriyama et al., *Bio/Technology* 6: 10 (1988); Zhang et al., *Plant Cell Rep.* 7: 379 (1988); Luo and Wu, *Plant Mol. Biol. Rep.* 6: 165 (1988); Zhang and Wu, *Theor. Appl. Genet.* 76: 835 (1988); Christou et al., *Bio/Technology* 9: 957 (1991)); rye (*Secale cereale*; De la Pena et al., *Nature* 325: 274 (1987)); sorghum (*Sorghum bicolor*; Cassas et al., *Proc. Natl. Acad. Sci. USA* 90: 11212 (1993)); sugar cane (*Saccharum* spp.; Bower and Birch, *Plant J.* 2: 409 (1992)); tall fescue (*Festuca arundinacea*; Wang et al., *Bio/Technology* 10: 691 (1992)); turfgrass (*Agrostis palustris*; Zhong et al., *Plant Cell Rep.* 13: 1 (1993)); wheat (*Triticum aestivum*; Vasil et al., *Bio/Technology* 10: 667 (1992); Weeks et al., *Plant Physiol.* 102: 1077 (1993); Becker et al., *Plant J.* 5: 299 (1994)), and alfalfa (Masoud, S.A. et al., *Transgen. Res.* 5: 313 (1996)).

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EXAMPLE 23: Host plants

Particularly useful plants for PHA production include those that produce carbon substrates which can be employed for PHA biosynthesis, including tobacco, wheat, potato, *Arabidopsis*, and high oil seed plants such as corn, soybean, canola, oil seed rape, sunflower, flax, peanut, sugarcane, switchgrass, and alfalfa.

If the host plant of choice does not produce the requisite fatty acid substrates in sufficient quantities, it can be modified, for example by mutagenesis or genetic transformation, to block or modulate the glycerol ester and fatty acid biosynthesis or degradation pathways so that it accumulates the appropriate substrates for PHA production. Expression of enzymes such as acyl-ACP thioesterase, fatty acyl hydroxylase, and yeast multifunctional protein (MFP) may serve to increase the flux of substrates in the peroxisome, leading to higher levels of PHA biosynthesis.

EXAMPLE 24: Nucleic acid mutation and hybridization

Variations in the nucleic acid sequence encoding a fusion protein may lead to mutant protein sequences that display equivalent or superior enzymatic characteristics when compared to the sequences disclosed herein. This invention accordingly encompasses nucleic acid sequences which are similar to the sequences disclosed herein, protein sequences which are similar to the sequences disclosed herein, and the nucleic acid sequences that encode them. Mutations may include deletions, insertions, truncations, substitutions, fusions, and the like.

Mutations to a nucleic acid sequence may be introduced in either a specific or random manner, both of which are well known to those of skill in the art of molecular biology. A myriad of site-directed mutagenesis techniques exist, typically using oligonucleotides to introduce mutations at specific locations in a nucleic acid sequence. Examples include single strand rescue (Kunkel, T. *Proc. Natl. Acad. Sci. U.S.A.*, 82: 488-492 (1985)), unique site elimination (Deng and Nickloff, *Anal. Biochem.* 200: 81 (1992)),



nick protection (Vandeyar, et al. *Gene* 65: 129-133 (1988)), and PCR (Costa, et al. *Methods Mol. Biol.* 57: 31-44 (1996)). Random or non-specific mutations may be generated by chemical agents (for a general review, see Singer and Kusmierk, *Ann. Rev. Biochem.* 52: 655-693 (1982)) such as nitrosoguanidine (Cerde-Olmedo et al., *J. Mol. Biol.* 33:705-719 (1968); Guerola, et al. *Nature New Biol.* 230: 122-125 (1971)) and 2-aminopurine (Rogan and Bessman, *J. Bacteriol.* 103: 622-633 (1970)), or by biological methods such as passage through mutator strains (Greener et al. *Mol. Biotechnol.* 7: 189-195 (1997)).

Nucleic acid hybridization is a technique well known to those of skill in the art of DNA manipulation. The hybridization properties of a given pair of nucleic acids is an indication of their similarity or identity. Mutated nucleic acid sequences may be selected for their similarity to the disclosed nucleic acid sequences on the basis of their hybridization to the disclosed sequences. Low stringency conditions may be used to select sequences with multiple mutations. One may wish to employ conditions such as about 0.15 M to about 0.9 M sodium chloride, at temperatures ranging from about 20°C to about 55°C. High stringency conditions may be used to select for nucleic acid sequences with higher degrees of identity to the disclosed sequences. Conditions employed may include about 0.02 M to about 0.15 M sodium chloride, about 0.5% to about 5% casein, about 0.02% SDS and/or about 0.1% N-laurylsarcosine, about 0.001 M to about 0.03 M sodium citrate, at temperatures between about 50°C and about 70°C. More preferably, high stringency conditions are 0.02 M sodium chloride, 0.5% casein, 0.02% SDS, 0.001 M sodium citrate, at a temperature of 50°C.

#### EXAMPLE 25: Determination of homologous and degenerate nucleic acid sequences

Modification and changes may be made in the sequence of the proteins of the present invention and the nucleic acid segments which encode them and still obtain a functional molecule that encodes a protein with desirable properties. The following is a discussion based upon changing the amino acid sequence of a protein to create an equivalent, or possibly an improved, second-generation molecule. The amino acid changes

may be achieved by changing the codons of the nucleic acid sequence, according to the codons given in Table 11.

Table 11: Codon degeneracies of amino acids

Amino acid	One letter	Three letter	Codons
Alanine	A	Ala	GCA GCC GCG GCT
Cysteine	C	Cys	TGC TGT
Aspartic acid	D	Asp	GAC GAT
Glutamic acid	E	Glu	GAA GAG
Phenylalanine	F	Phe	TTC TTT
Glycine	G	Gly	GGA GGC GGG GGT
Histidine	H	His	CAC CAT
Isoleucine	I	Ile	ATA ATC ATT
Lysine	K	Lys	AAA AAG
Leucine	L	Leu	TTA TTG CTA CTC CTG CTT
Methionine	M	Met	ATG
Asparagine	N	Asn	AAC AAT
Proline	P	Pro	CCA CCC CCG CCT
Glutamine	Q	Gln	CAA CAG
Arginine	R	Arg	AGA AGG CGA CGC CGG CGT
Serine	S	Ser	AGC AGT TCA TCC TCG TCT
Threonine	T	Thr	ACA ACC ACG ACT
Valine	V	Val	GTA GTC GTG GTT
Tryptophan	W	Trp	TGG
Tyrosine	Y	Tyr	TAC TAT

- 5 Certain amino acids may be substituted for other amino acids in a protein sequence without appreciable loss of enzymatic activity. It is thus contemplated that various changes may be made in the peptide sequences of the disclosed protein sequences, or their corresponding nucleic acid sequences without appreciable loss of the biological activity.

- 10 In making such changes, the hydropathic index of amino acids may be considered. The importance of the hydropathic amino acid index in conferring interactive biological function on a protein is generally understood in the art (Kyte and Doolittle, *J. Mol. Biol.* 157: 105-132 (1982)). It is accepted that the relative hydropathic character of the amino acid contributes to the secondary structure of the resultant protein, which in turn defines th

interaction of the protein with other molecules, for example, enzymes, substrates, receptors, DNA, antibodies, antigens, and the like.

Each amino acid has been assigned a hydropathic index on the basis of their hydrophobicity and charge characteristics. These are: isoleucine (+4.5); valine (+4.2);  
5 leucine (+3.8); phenylalanine (+2.8); cysteine/cystine (+2.5); methionine (+1.9); alanine (+1.8); glycine (-0.4); threonine (-0.7); serine (-0.8); tryptophan (-0.9); tyrosine (-1.3); proline (-1.6); histidine (-3.2); glutamate/glutamine/aspartate/asparagine (-3.5); lysine (-3.9); and arginine (-4.5).

It is known in the art that certain amino acids may be substituted by other amino  
10 acids having a similar hydropathic index or score and still result in a protein with similar biological activity, i.e., still obtain a biologically functional protein. In making such changes, the substitution of amino acids whose hydropathic indices are within  $\pm 2$  is preferred, those within  $\pm 1$  are more preferred, and those within  $\pm 0.5$  are most preferred.

It is also understood in the art that the substitution of like amino acids may be made  
15 effectively on the basis of hydrophilicity. U.S. Patent No. 4,554,101 (Hopp, T.P., issued November 19, 1985) states that the greatest local average hydrophilicity of a protein, as governed by the hydrophilicity of its adjacent amino acids, correlates with a biological property of the protein. The following hydrophilicity values have been assigned to amino acids: arginine/lysine (+3.0); aspartate/glutamate (+3.0  $\pm 1$ ); serine (+0.3);  
20 asparagine/glutamine (+0.2); glycine (0); threonine (-0.4); proline (-0.5  $\pm 1$ ); alanine/histidine (-0.5); cysteine (-1.0); methionine (-1.3); valine (-1.5); leucine/isoleucine (-1.8); tyrosine (-2.3); phenylalanine (-2.5); and tryptophan (-3.4).

It is understood that an amino acid may be substituted by another amino acid having a similar hydrophilicity score and still result in a protein with similar biological activity, i.e.,  
25 still obtain a biologically functional protein. In making such changes, the substitution of amino acids whose hydropathic indices are within  $\pm 2$  is preferred, those within  $\pm 1$  are more preferred, and those within  $\pm 0.5$  are most preferred.

As outlined above, amino acid substitutions are therefore based on the relative similarity of the amino acid side-chain substituents, for example, their hydrophobicity, hydrophilicity, charge, size, and the like. Exemplary substitutions which take various of the foregoing characteristics into consideration are well known to those of skill in the art and include: arginine and lysine; glutamate and aspartate; serine and threonine; glutamine and asparagine; and valine, leucine, and isoleucine. Changes which are not expected to be advantageous may also be used if these resulted in functional fusion proteins.

All of the compositions and/or methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the compositions and methods of this invention have been described in terms of preferred embodiments, it will be apparent to those of skill in the art that variations may be applied to the compositions and/or methods and in the steps or in the sequence of steps of the methods described herein without departing from the concept, spirit and scope of the invention. More specifically, it will be apparent that certain agents which are both chemically and physiologically related may be substituted for the agents described herein while the same or similar results would be achieved. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the invention.

## SEQUENCE LISTING

## SEQUENCE LISTING

## 5 (1) GENERAL INFORMATION:

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(E) COUNTRY: Switzerland  
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(ii) TITLE OF INVENTION: BIOSYNTHESIS OF MEDIUM CHAIN LENGTH  
POLYHYDROXYALKANOATES

(iii) NUMBER OF SEQUENCES: 26

30

## (iv) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk  
(B) COMPUTER: IBM PC compatible  
(C) OPERATING SYSTEM: PC-DOS/MS-DOS  
35 (D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

## (2) INFORMATION FOR SEQ ID NO: 1:

## 40 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1677 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
45 (D) TOPOLOGY: linear

## 50 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

ATGAGTCAGA AGAACAATAA CGAGCTTCCC AAGCAAGCCG CGGAAAACAC GCTGAACCTG 60  
AATCCGGTGA TCGGCATCCG GGGCAAGGAC CTGCTCACCT CCGCGCGCAT GGTCCTGCTC 120  
55 CAGGCGGTGC GCCAGCCGCT GCACAGCGCC AGGCACGTGG CGCATTTCAG CCTGGAGCTG 180

	AAGAACGTCC TGCTCGGCCA GTCGGAGCTA CGCCCAGGCG ATGACGACCG ACGCTTTTCC	240
	GATCCGGCCT GGAGCCAGAA TCCACTGTAC AAGCGCTACA TGCAGACCTA CCTGGCCTGG	300
5	CGCAAGGAGC TGCACAGCTG GATCAGCCAC AGCGACCTGT CGCCGCAGGA CATCAGTCGT	360
	GGCCAGTTTCG TCATCAACCT GCTGACCGAG GCGATGTCGC CGACCAACAG CCTGAGCAAC	420
10	CCGGCGGCGG TCAAGCGCTT CTTGAGACC GCGGCAAGA GCCTGCTGGA CGGCCTCGGC	480
	CACCTGGCCA AGGACCTGGT GAACAACGGC GGGATGCCGA GCCAGGTGGA CATGGACGCC	540
	TTCGAGGTGG GCAAGAACCT GGCCACCACC GAGGGCGCCG TGGTGTTCGG CAACGACGTG	600
15	CTGGAAGTGA TCCAGTACCG GCCGATCACC GAGTCGGTGC ACGAACGCCC GCTGCTGGTG	660
	GTGCCGCCGC AGATCAACAA GTTCTACGTC TTCGACCTGT CGCCGGACAA GAGCCTGGCG	720
20	CGCTTCTGCC TGCGCAACGG CGTGCAGACC TTCATCGTCA GTTGGCGCAA CCCGACCAAG	780
	TCGCAGCGCG AATGGGGCCT GACCACCTAT ATCGAGGCGC TCAAGGAGGC CATCGAGGTA	840
	GTCCTGTCGA TCACCGGCAG CAAGGACCTC AACCTCCTCG GCGCCTGCTC CGGCGGGATC	900
25	ACCACCGCGA CCCTGGTCGG CCACTACGTG GCCAGCGGCG AGAAGAAGGT CAACGCCTTC	960
	ACCCAACTGG TCAGCGTGCT CGACTTCGAA CTGAATACCC AGGTCGCGCT GTTCGCCGAC	1020
30	GAGAAGACTC TGGAGGCCGC CAAGCGTCGT TCCTACCAGT CCGGCGTGCT GGAGGGCAAG	1080
	GACATGGCCA AGGTGTTCGC CTGGATGCGC CCCAACGACC TGATCTGGAA CTACTGGGTC	1140
	AACAAGTACC TGCTCGGCAA CCAGCCGCCG GCGTTCGACA TCCTCTACTG GAACAACGAC	1200
35	ACCACGCGCC TGCCCGCCGC GCTGCACGGC GAGTTCGTCG AACTGTTCAA GAGCAACCCG	1260
	CTGAACCGCC CCGGCGCCCT GGAGGTCTCC GGCACGCCCA TCGACCTGAA GCAGGTGACT	1320
40	TGCGACTTCT ACTGTGTCGC CGGTCTGAAC GACCACATCA CCCCTGGGA GTCGTGCTAC	1380
	AAGTCGGCCA GGCTGCTGGG TGGCAAGTGC GAGTTCATCC TCTCCAACAG CGGTCACATC	1440
	CAGAGCATCC TCAACCCACC GGGCAACCCC AAGGCACGCT TCATGACCAA TCCGGAAGTG	1500
45	CCCGCCGAGC CCAAGGCCTG GCTGGAACAG GCCGGCAAGC ACGCCGACTC GTGGTGGTTG	1560
	CACTGGCAGC AATGGCTGGC CGAACGCTCC GGCAAGACCC GCAAGGCGCC CGCCAGCCTG	1620
50	GGCAACAAGA CCTATCCGGC CGGCGAAGCC GCGCCCGGAA CCTACGTGCA TGAACGA	1677

## (2) INFORMATION FOR SEQ ID NO: 2:

## (i) SEQUENCE CHARACTERISTICS:

- 55 (A) LENGTH: 559 amino acids  
(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

5	Met	Ser	Gln	Lys	Asn	Asn	Asn	Glu	Leu	Pro	Lys	Gln	Ala	Ala	Glu	Asn	1	5	10	15
	Thr	Leu	Asn	Leu	Asn	Pro	Val	Ile	Gly	Ile	Arg	Gly	Lys	Asp	Leu	Leu	20	25	30	
10	Thr	Ser	Ala	Arg	Met	Val	Leu	Leu	Gln	Ala	Val	Arg	Gln	Pro	Leu	His	35	40	45	
	Ser	Ala	Arg	His	Val	Ala	His	Phe	Ser	Leu	Glu	Leu	Lys	Asn	Val	Leu	50	55	60	
15	Leu	Gly	Gln	Ser	Glu	Leu	Arg	Pro	Gly	Asp	Asp	Asp	Arg	Arg	Phe	Ser	65	70	75	80
	Asp	Pro	Ala	Trp	Ser	Gln	Asn	Pro	Leu	Tyr	Lys	Arg	Tyr	Met	Gln	Thr	85	90	95	
20	Tyr	Leu	Ala	Trp	Arg	Lys	Glu	Leu	His	Ser	Trp	Ile	Ser	His	Ser	Asp	100	105	110	
25	Leu	Ser	Pro	Gln	Asp	Ile	Ser	Arg	Gly	Gln	Phe	Val	Ile	Asn	Leu	Leu	115	120	125	
	Thr	Glu	Ala	Met	Ser	Pro	Thr	Asn	Ser	Leu	Ser	Asn	Pro	Ala	Ala	Val	130	135	140	
30	Lys	Arg	Phe	Phe	Glu	Thr	Gly	Gly	Lys	Ser	Leu	Leu	Asp	Gly	Leu	Gly	145	150	155	160
	His	Leu	Ala	Lys	Asp	Leu	Val	Asn	Asn	Gly	Gly	Met	Pro	Ser	Gln	Val	165	170	175	
35	Asp	Met	Asp	Ala	Phe	Glu	Val	Gly	Lys	Asn	Leu	Ala	Thr	Thr	Glu	Gly	180	185	190	
40	Ala	Val	Val	Phe	Arg	Asn	Asp	Val	Leu	Glu	Leu	Ile	Gln	Tyr	Arg	Pro	195	200	205	
	Ile	Thr	Glu	Ser	Val	His	Glu	Arg	Pro	Leu	Leu	Val	Val	Pro	Pro	Gln	210	215	220	
45	Ile	Asn	Lys	Phe	Tyr	Val	Phe	Asp	Leu	Ser	Pro	Asp	Lys	Ser	Leu	Ala	225	230	235	240
	Arg	Phe	Cys	Leu	Arg	Asn	Gly	Val	Gln	Thr	Phe	Ile	Val	Ser	Trp	Arg	245	250	255	
50	Asn	Pro	Thr	Lys	Ser	Gln	Arg	Glu	Trp	Gly	Leu	Thr	Thr	Tyr	Ile	Glu	260	265	270	
55	Ala	Leu	Lys	Glu	Ala	Ile	Glu	Val	Val	Leu	Ser	Ile	Thr	Gly	Ser	Lys				

	275	280	285
	Asp Leu Asn Leu Leu Gly	Ala Cys Ser Gly Gly	Ile Thr Thr Ala Thr
	290	295	300
5	Leu Val Gly His Tyr Val	Ala Ser Gly Glu Lys	Lys Val Asn Ala Phe
	305	310	315 320
	Thr Gln Leu Val Ser Val	Leu Asp Phe Glu Leu	Asn Thr Gln Val Ala
10		325	330 335
	Leu Phe Ala Asp Glu Lys	Thr Leu Glu Ala Ala	Lys Arg Arg Ser Tyr
	340	345	350
15	Gln Ser Gly Val Leu Glu	Gly Lys Asp Met Ala	Lys Val Phe Ala Trp
	355	360	365
	Met Arg Pro Asn Asp Leu	Ile Trp Asn Tyr Trp	Val Asn Asn Tyr Leu
20		370 375	380
	Leu Gly Asn Gln Pro Pro	Ala Phe Asp Ile Leu	Tyr Trp Asn Asn Asp
	385	390	395 400
	Thr Thr Arg Leu Pro Ala	Ala Leu His Gly Glu	Phe Val Glu Leu Phe
25		405	410 415
	Lys Ser Asn Pro Leu Asn	Arg Pro Gly Ala Leu	Glu Val Ser Gly Thr
	420	425	430
30	Pro Ile Asp Leu Lys Gln	Val Thr Cys Asp Phe	Tyr Cys Val Ala Gly
	435	440	445
	Leu Asn Asp His Ile Thr	Pro Trp Glu Ser Cys	Tyr Lys Ser Ala Arg
	450	455	460
35	Leu Leu Gly Gly Lys Cys	Glu Phe Ile Leu Ser	Asn Ser Gly His Ile
	465	470	475 480
	Gln Ser Ile Leu Asn Pro	Pro Gly Asn Pro Lys	Ala Arg Phe Met Thr
40		485	490 495
	Asn Pro Glu Leu Pro Ala	Glu Pro Lys Ala Trp	Leu Glu Gln Ala Gly
	500	505	510
45	Lys His Ala Asp Ser Trp	Trp Leu His Trp Gln	Gln Trp Leu Ala Glu
	515	520	525
	Arg Ser Gly Lys Thr Arg	Lys Ala Pro Ala Ser	Leu Gly Asn Lys Thr
	530	535	540
50	Tyr Pro Ala Gly Glu Ala	Ala Pro Gly Thr Tyr	Val His Glu Arg
	545	550	555

(2) INFORMATION FOR SEQ ID NO: 3:

55

(i) SEQUENCE CHARACTERISTICS:



(A) LENGTH: 1680 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

5

10 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

	ATGCGAGAAA AGCAGGAATC GGGTAGCGTG CCGGTGCCCC CCGAGTTCAT GAGTGCACAG	60
	AGCGCCATCG TCGGCCTGCG CGGCAAGGAC CTGCTGACGA CGGTCCGCAG CCTGGCTGTC	120
15	CACGGCCTGC GCCAGCCGCT GCACAGTGCG CGGCACCTGG TCGCCTTCGG AGGCCAGTTG	180
	GGCAAGGTGC TGCTGGGCGA CACCCTGCAC CAGCCGAACC CACAGGACGC CCGCTTCCAG	240
20	GATCCATCCT GGC GCCTCAA TCCCTTCTAC CGGCGCACCC TGCAGGCCTA CCTGGCGTGG	300
	CAGAAACAAC TGCTCGCCTG GATCGACGAA AGCAACCTGG ACTGCGACGA TCGCGCCCGC	360
	GCCCCGCTTC TCGTCGCCTT GCTCTCCGAC GCCGTGGCAC CCAGCAACAG CCTGATCAAT	420
25	CCACTGGCGT TAAAGGAACT GTTCAATACC GGCGGGATCA GCCTGCTCAA TGGCGTCCGC	480
	CACCTGCTCG AAGACCTGGT GCACAACGGC GGCATGCCCA GCCAGGTGAA CAAGACCGCC	540
30	TTCGAGATCG GTCGCAACCT CGCCACCACG CAAGGCGCGG TGGTGTTCGG CAACGAGGTG	600
	CTGGAGCTGA TCCAGTACAA GCCGCTGGGC GAGCGCCAGT ACGCCAAGCC CCTGCTGATC	660
	GTGCCGCCGC AGATCAACAA GTACTACATC TTCGACCTGT CGCCGGAAAA GAGCTTCGTC	720
35	CAGTACGCCC TGAAGAACAA CCTGCAGGTC TTCGTCATCA GTTGGCGCAA CCCCAGCGCC	780
	CAGCACC GCG AATGGGGCCT GAGCACCTAT GTCGAGGCCC TCGACCAGGC CATCGAGGTC	840
40	AGCCGCGAGA TCACCGGCAG CCGCAGCGTG AACCTGGCCG GCGCCTGCGC CGGCGGGCTC	900
	ACCGTAGCCG CCTTGCTCGG CCACCTGCAG GTGCGCCGGC AACTGCGCAA GGTCAGTAGC	960
	GTCACCTACC TGGTCAGCCT GCTCGACAGC CAGATGGAAA GCCCGGCGAT GCTCTTCGCC	1020
45	GACGAGCAGA CCCTGGAGAG CAGCAAGCGC CGCTCCTACC AGCATGGCGT GCTGGACGGG	1080
	CGCGACATGG CCAAGGTGTT CGCCTGGATG CGCCCCAACG ACCTGATCTG GAACTACTGG	1140
50	GTCAACAACCT ACCTGCTCGG CAGGCAGCCG CCGGCGTTTCG ACATCCTCTA CTGGAACAAC	1200
	GACAACACGC GGCTGCCCCG GCGGTTCCAC GGCGAACTGC TCGACCTGTT CAAGCACAAC	1260
	CCGCTGACCC GCCCGGGCGC GCTGGAGGTC AGCGGGACCG CCGTGGACCT GGGCAAGGTG	1320
55	GCGATCGACA GCTTCCACGT CGCCGGCATC ACCGACCACA TCACGCCCTG GGACGCGGTG	1380

TATCGCTCGG CCCTCCTGCT GGGCGGCCAG CGCCGCTTCA TCCTGTCCAA CAGCGGGCAC 1440  
 ATCCAGAGCA TCCTCAACCC TCCCGGAAAC CCCAAGGCCT GCTACTTCGA GAACGACAAG 1500  
 5 CTGAGCAGCG ATCCACGCGC CTGGTACTAC GACGCCAAGC GCGAAGAGGG CAGCTGGTGG 1560  
 CCGGTCTGGC TGGGCTGGCT GCAGGAGCGC TCGGGCGAGC TGGGCAACCC TGACTTCAAC 1620  
 10 CTTGGCAGCG CCGCGCATCC GCCCCTCGAA GCGGCCCCGG GCACCTACGT GCATATACGC 1680

## (2) INFORMATION FOR SEQ ID NO: 4:

15 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 560 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS:  
 (D) TOPOLOGY: linear

## 25 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

Met Arg Glu Lys Gln Glu Ser Gly Ser Val Pro Val Pro Ala Glu Phe  
 1 5 10 15  
 30 Met Ser Ala Gln Ser Ala Ile Val Gly Leu Arg Gly Lys Asp Leu Leu  
 20 25 30  
 Thr Thr Val Arg Ser Leu Ala Val His Gly Leu Arg Gln Pro Leu His  
 35 35 40 45  
 40 Ser Ala Arg His Leu Val Ala Phe Gly Gly Gln Leu Gly Lys Val Leu  
 50 55 60  
 Leu Gly Asp Thr Leu His Gln Pro Asn Pro Gln Asp Ala Arg Phe Gln  
 65 70 75 80  
 Asp Pro Ser Trp Arg Leu Asn Pro Phe Tyr Arg Arg Thr Leu Gln Ala  
 85 90 95  
 45 Tyr Leu Ala Trp Gln Lys Gln Leu Leu Ala Trp Ile Asp Glu Ser Asn  
 100 105 110  
 Leu Asp Cys Asp Asp Arg Ala Arg Ala Arg Phe Leu Val Ala Leu Leu  
 115 120 125  
 50 Ser Asp Ala Val Ala Pro Ser Asn Ser Leu Ile Asn Pro Leu Ala Leu  
 130 135 140  
 Lys Glu Leu Phe Asn Thr Gly Gly Ile Ser Leu Leu Asn Gly Val Arg  
 145 150 155 160

	His	Leu	Leu	Glu	Asp	Leu	Val	His	Asn	Gly	Gly	Met	Pro	Ser	Gln	Val	
						165				170					175		
5	Asn	Lys	Thr	Ala	Phe	Glu	Ile	Gly	Arg	Asn	Leu	Ala	Thr	Thr	Gln	Gly	
						180				185					190		
	Ala	Val	Val	Phe	Arg	Asn	Glu	Val	Leu	Glu	Leu	Ile	Gln	Tyr	Lys	Pro	
						195			200				205				
10	Leu	Gly	Glu	Arg	Gln	Tyr	Ala	Lys	Pro	Leu	Leu	Ile	Val	Pro	Pro	Gln	
						210			215				220				
	Ile	Asn	Lys	Tyr	Tyr	Ile	Phe	Asp	Leu	Ser	Pro	Glu	Lys	Ser	Phe	Val	
						225			230			235				240	
15	Gln	Tyr	Ala	Leu	Lys	Asn	Asn	Leu	Gln	Val	Phe	Val	Ile	Ser	Trp	Arg	
						245				250					255		
	Asn	Pro	Asp	Ala	Gln	His	Arg	Glu	Trp	Gly	Leu	Ser	Thr	Tyr	Val	Glu	
20						260			265					270			
	Ala	Leu	Asp	Gln	Ala	Ile	Glu	Val	Ser	Arg	Glu	Ile	Thr	Gly	Ser	Arg	
						275			280				285				
25	Ser	Val	Asn	Leu	Ala	Gly	Ala	Cys	Ala	Gly	Gly	Leu	Thr	Val	Ala	Ala	
						290			295			300					
	Leu	Leu	Gly	His	Leu	Gln	Val	Arg	Arg	Gln	Leu	Arg	Lys	Val	Ser	Ser	
						305			310			315				320	
30	Val	Thr	Tyr	Leu	Val	Ser	Leu	Leu	Asp	Ser	Gln	Met	Glu	Ser	Pro	Ala	
						325				330					335		
	Met	Leu	Phe	Ala	Asp	Glu	Gln	Thr	Leu	Glu	Ser	Ser	Lys	Arg	Arg	Ser	
35						340			345					350			
	Tyr	Gln	His	Gly	Val	Leu	Asp	Gly	Arg	Asp	Met	Ala	Lys	Val	Phe	Ala	
						355			360				365				
40	Trp	Met	Arg	Pro	Asn	Asp	Leu	Ile	Trp	Asn	Tyr	Trp	Val	Asn	Asn	Tyr	
						370			375				380				
	Leu	Leu	Gly	Arg	Gln	Pro	Pro	Ala	Phe	Asp	Ile	Leu	Tyr	Trp	Asn	Asn	
						385			390			395			400		
45	Asp	Asn	Thr	Arg	Leu	Pro	Ala	Ala	Phe	His	Gly	Glu	Leu	Leu	Asp	Leu	
						405				410					415		
	Phe	Lys	His	Asn	Pro	Leu	Thr	Arg	Pro	Gly	Ala	Leu	Glu	Val	Ser	Gly	
50						420			425				430				
	Thr	Ala	Val	Asp	Leu	Gly	Lys	Val	Ala	Ile	Asp	Ser	Phe	His	Val	Ala	
						435			440				445				
55	Gly	Ile	Thr	Asp	His	Ile	Thr	Pro	Trp	Asp	Ala	Val	Tyr	Arg	Ser	Ala	
						450			455				460				

Leu Leu Leu Gly Gly Gln Arg Arg Phe Ile Leu Ser Asn Ser Gly His  
465 470 475 480

5 Ile Gln Ser Ile Leu Asn Pro Pro Gly Asn Pro Lys Ala Cys Tyr Phe  
485 490 495

Glu Asn Asp Lys Leu Ser Ser Asp Pro Arg Ala Trp Tyr Tyr Asp Ala  
500 505 510

10 Lys Arg Glu Glu Gly Ser Trp Trp Pro Val Trp Leu Gly Trp Leu Gln  
515 520 525

Glu Arg Ser Gly Glu Leu Gly Asn Pro Asp Phe Asn Leu Gly Ser Ala  
15 530 535 540

Ala His Pro Pro Leu Glu Ala Ala Pro Gly Thr Tyr Val His Ile Arg  
545 550 555 560

20

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 29 base pairs  
25 (B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

30

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

35 GGAGAATTCC CGATGAGCCA GAAGAACAA

29

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:  
40 (A) LENGTH: 29 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

45

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

50

CTGGAAGCTT TTGATCGTTC ATGCACGTA

29

(2) INFORMATION FOR SEQ ID NO: 7:

55 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 29 base pairs

- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

5

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

GTGGAATTCA TGC GTGAAAA GCAGGAATC

29

10

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 29 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

15

20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

GGCCAAGCTT TTGAGCGTAT ATGCACGTA

29

25

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1731 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

30

35

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

40	ATGGCTGCAT CTTTCTCTGT CCCCTCTATG ATCATGGAAG AGGAAGGGAG ATTTGAGGCG	60
	GAAGTTGCGG AAGTGCAGAC TTGGTGGAGC TCAGAGAGGT TCAAGCTAAC AAGGCGTCCT	120
	TACACGGCCC GTGACGTGGT GGCTCTACGT GGTCATCTCA AGCAAGGTTA TGCTTCGAAC	180
45	GAGATGGCTA AGAAGCTGTG GAGAACGCTC AAGAGTCACC AAGTCAACGG CACGGCGTCT	240
	CGCACGTTTG GTGCCTTGGA CCCTGTTTCTG GTGACAATGA TGGCTAAACA TTTAGACACC	300
50	ATTTATGTCT CTGGTTGGCA GTGCTCGTCT ACTCACACCT CCACTAACGA GCCTGGTCCG	360
	GATCTTGCTG ACTATCCATA CGATACCGTT CCTAACAAGG TCGAACATCT CTTCTTCGCT	420
	CAGCAGTACC ATGACAGAAA ACAGAGGGAG GCGAGAATGA GCATGAGCAG AGAAGAAAGA	480
55	GCAAAAACCTC CGTTTGTGGA CTACTTGAAG CCCATCATCG CCGACGGAGG AACCGGCTTC	540

GGCGGTACCA CTGCCACCGT AAAACTCTGC AAACCTCTTCG TTGAAAGAGG AGCCGCTGGG 600  
 GTCCACATCG AGGACCAGTC CTCCGTCACC AAGAAGTGTG GCCACATGGC CGGAAAAGTC 660  
 5 CTCGTGGCAG TCAGTGAACA CATCAACCGC CTTGTTGCGG CTCGGCTCCA GTTCGACGTG 720  
 ATGGGCACAG AGACCGTCCT GGTCGCTAGA ACGGACGCGG TCGCGCCAC TCTGATCCAA 780  
 10 TCGAACATTG ACTCAAGGGA CCACCAGTTC ATCCTCGGTG TACTAACCC AACCTTAGA 840  
 GGCAAGAGTT TGTCTCGCT TCTGGCCGAG GGAATGGCTG TAGGCAATAA TGGTCCAGCG 900  
 TTGCAAGCGA TTGAGGATCA ATGGCTTAGC TCAGCTCGTC TCATGACTTT CTCGGACGCT 960  
 15 GTCGTGGAGG CTCTCAAGCG CATGAACCTA AGTGAGAATG AGAAGAGCCG GAGAGTGACC 1020  
 GAGTGGCTAA TCCATGCAAG GTACGAGAAC TGCCTTTCAA ACGAGCAAGG CCGAGAATTA 1080  
 20 GCAGCAAAAC TCGGTGTGAC TGATCTTTTC TGGGACTGGG ACTTGCCCAG AACCAGAGAA 1140  
 GGATTCTACC GGTTCCAAGG CTCGGTCACA GCAGCCGTGG TCCGTGGCTG GGCCTTTGCA 1200  
 CAGATAGCTG ATCTCATCTG GATGGAAACC GCAAGCCCTG ACCTCAACGA ATGCACCCAA 1260  
 25 TTCGCAGAAG GAGTCAAGTC CAAGACACCA GAGGTAATGC TCGCCTACAA CCTCTCCCCA 1320  
 TCCTTCAACT GGGACGCTTC TGGTATGACG GATCAGCAGA TGATGGAGTT CATTCCACGA 1380  
 30 ATCGCCAGGC TCGGTTATTG CTGGCAGTTT ATAACCCTTG CGGGTTTCCA TGCGGATGCT 1440  
 CTTGTGGTCG ATACGTTTGC AAAGGATTAC GCGAGGAGAG GGATGCTGGC TTATGTCGAG 1500  
 AGGATACAGA GAGAAGAGAG GAGCAATGGG GTTGACACAT TGGCTCATCA GAAATGGTCA 1560  
 35 GGTGCTAATT ACTATGATCG TTATCTTAAG ACCGTCCAAG GTGGAATCTC CTCCACTGCA 1620  
 GCCATGGGCA AAGGTGTTAC CGAGGAACAA TTCAAAGAGA CCTGGACGAG GCCGGGAGCT 1680  
 40 GCTGGAATGG GCGAAGGGAC TAGCCTTGTG GTGGCCAAGT CCAGAATGTA A 1731

## (2) INFORMATION FOR SEQ ID NO: 10:

## (i) SEQUENCE CHARACTERISTICS:

- 45 (A) LENGTH: 576 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS:  
 (D) TOPOLOGY: linear

50

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

55

Met Ala Ala Ser Phe Ser Val Pro Ser Met Ile Met Glu Glu Glu Gly

	1		5		10		15									
	Arg	Phe	Glu	Ala	Glu	Val	Ala	Glu	Val	Gln	Thr	Trp	Trp	Ser	Ser	Glu
			20					25						30		
5	Arg	Phe	Lys	Leu	Thr	Arg	Arg	Pro	Tyr	Thr	Ala	Arg	Asp	Val	Val	Ala
			35					40					45			
	Leu	Arg	Gly	His	Leu	Lys	Gln	Gly	Tyr	Ala	Ser	Asn	Glu	Met	Ala	Lys
10			50				55					60				
	Lys	Leu	Trp	Arg	Thr	Leu	Lys	Ser	His	Gln	Val	Asn	Gly	Thr	Ala	Ser
	65					70				75						80
15	Arg	Thr	Phe	Gly	Ala	Leu	Asp	Pro	Val	Gln	Val	Thr	Met	Met	Ala	Lys
					85					90					95	
	His	Leu	Asp	Thr	Ile	Tyr	Val	Ser	Gly	Trp	Gln	Cys	Ser	Ser	Thr	His
			100						105					110		
20	Thr	Ser	Thr	Asn	Glu	Pro	Gly	Pro	Asp	Leu	Ala	Asp	Tyr	Pro	Tyr	Asp
			115					120					125			
	Thr	Val	Pro	Asn	Lys	Val	Glu	His	Leu	Phe	Phe	Ala	Gln	Gln	Tyr	His
25			130				135					140				
	Asp	Arg	Lys	Gln	Arg	Glu	Ala	Arg	Met	Ser	Met	Ser	Arg	Glu	Glu	Arg
	145					150				155						160
30	Ala	Lys	Thr	Pro	Phe	Val	Asp	Tyr	Leu	Lys	Pro	Ile	Ile	Ala	Asp	Gly
					165				170						175	
	Gly	Thr	Gly	Phe	Gly	Gly	Thr	Thr	Ala	Thr	Val	Lys	Leu	Cys	Lys	Leu
			180					185					190			
35	Phe	Val	Glu	Arg	Gly	Ala	Ala	Gly	Val	His	Ile	Glu	Asp	Gln	Ser	Ser
			195					200					205			
	Val	Thr	Lys	Lys	Cys	Gly	His	Met	Ala	Gly	Lys	Val	Leu	Val	Ala	Val
40			210				215					220				
	Ser	Glu	His	Ile	Asn	Arg	Leu	Val	Ala	Ala	Arg	Leu	Gln	Phe	Asp	Val
	225				230						235				240	
45	Met	Gly	Thr	Glu	Thr	Val	Leu	Val	Ala	Arg	Thr	Asp	Ala	Val	Ala	Pro
					245				250						255	
	Thr	Leu	Ile	Gln	Ser	Asn	Ile	Asp	Ser	Arg	Asp	His	Gln	Phe	Ile	Leu
			260					265					270			
50	Gly	Val	Thr	Asn	Pro	Asn	Leu	Arg	Gly	Lys	Ser	Leu	Ser	Ser	Leu	Leu
			275				280					285				
	Ala	Glu	Gly	Met	Ala	Val	Gly	Asn	Asn	Gly	Pro	Ala	Leu	Gln	Ala	Ile
55			290				295					300				

Glu Asp Gln Trp Leu Ser Ser Ala Arg Leu Met Thr Phe Ser Asp Ala  
 305 310 315 320  
 Val Val Glu Ala Leu Lys Arg Met Asn Leu Ser Glu Asn Glu Lys Ser  
 5 325 330 335  
 Arg Arg Val Thr Glu Trp Leu Ile His Ala Arg Tyr Glu Asn Cys Leu  
 340 345 350  
 Ser Asn Glu Gln Gly Arg Glu Leu Ala Ala Lys Leu Gly Val Thr Asp  
 10 355 360 365  
 Leu Phe Trp Asp Trp Asp Leu Pro Arg Thr Arg Glu Gly Phe Tyr Arg  
 15 370 375 380  
 Phe Gln Gly Ser Val Thr Ala Ala Val Val Arg Gly Trp Ala Phe Ala  
 385 390 395 400  
 Gln Ile Ala Asp Leu Ile Trp Met Glu Thr Ala Ser Pro Asp Leu Asn  
 20 405 410 415  
 Glu Cys Thr Gln Phe Ala Glu Gly Val Lys Ser Lys Thr Pro Glu Val  
 420 425 430  
 Met Leu Ala Tyr Asn Leu Ser Pro Ser Phe Asn Trp Asp Ala Ser Gly  
 25 435 440 445  
 Met Thr Asp Gln Gln Met Met Glu Phe Ile Pro Arg Ile Ala Arg Leu  
 450 455 460  
 Gly Tyr Cys Trp Gln Phe Ile Thr Leu Ala Gly Phe His Ala Asp Ala  
 30 465 470 475 480  
 Leu Val Val Asp Thr Phe Ala Lys Asp Tyr Ala Arg Arg Gly Met Leu  
 35 485 490 495  
 Ala Tyr Val Glu Arg Ile Gln Arg Glu Glu Arg Ser Asn Gly Val Asp  
 500 505 510  
 Thr Leu Ala His Gln Lys Trp Ser Gly Ala Asn Tyr Tyr Asp Arg Tyr  
 40 515 520 525  
 Leu Lys Thr Val Gln Gly Gly Ile Ser Ser Thr Ala Ala Met Gly Lys  
 530 535 540  
 Gly Val Thr Glu Glu Gln Phe Lys Glu Thr Trp Thr Arg Pro Gly Ala  
 45 545 550 555 560  
 Ala Gly Met Gly Glu Gly Thr Ser Leu Val Val Ala Lys Ser Arg Met  
 50 565 570 575

## (2) INFORMATION FOR SEQ ID NO: 11:

- 55 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 26 base pairs



- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

5

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

10

ACTGAAGCTT TGGGCAAAGG TGTTAC

26

(2) INFORMATION FOR SEQ ID NO: 12:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 28 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

GTGGTCTAGA AGTTTTTCTG CGAAGATG

28

(2) INFORMATION FOR SEQ ID NO: 13:

30

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 102 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

35

40

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

GGCAAAGGTG TTACCGAGGA ACAATTCAAA GAGACCTGGA CGAGGCCGGG AGCTGCTGGA

60

ATGGGCGAAG GGACTAGCCT TGTGGTGGCC AAGTCCAGAA TG

102

(2) INFORMATION FOR SEQ ID NO: 14:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 34 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS:
  - (D) TOPOLOGY: linear

50

55

[illegible]

15 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1677 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

	ATGAGCCAGA	AGAACAATAA	CGAGCTTCCC	AAGCAAGCCG	CGGAAAACAC	GCTGAACCTG	60
	AATCCGGTGA	TCGGCATCCG	GGGCAAGGAC	CTGTCTCACCT	CCGCGCGCAT	GGTCCTGCTC	120
30	CAGGCGGTGC	GCCAGCCGCT	GCACAGCGCC	AGGCACGTGG	CGCATTTCAG	CCTGGAGCTG	180
	AAGAACGTCC	TGCTCGGCCA	GTCGGAGCTA	CGCCCAGGCG	ATGACGACCG	ACGCTTTTCC	240
35	GATCCGGCCT	GGAGCCAGAA	TCCACTGTAC	AAGCGCTACA	TGCAGACCTA	CCTGGCCTGG	300
	CGCAAGGAGC	TGCACAGCTG	GATCAGCCAC	AGCGACCTGT	CGCCGCAGGA	CATCAGTCGT	360
	GGCCAGTTCG	TCATCAACCT	GCTGACCGAG	GCGATGTCGC	CGACCAACAG	CCTGAGCAAC	420
40	CCGGCGGCGG	TCAAGCGCTT	CTTCGAGACC	GGCGGCAAGA	GCCTGCTGGA	CGGCCTCGGC	480
	CACCTGGCCA	AGGACCTGGT	GAACAACGGC	GGGATGCCGA	GCCAGGTGGA	CATGGACGCC	540
45	TTCGAGGTGG	GCAAGAACCT	GGCCACCACC	GAGGGCGCCG	TGGTGTTCGG	CAACGACGTG	600
	CTGGAActGA	TCCAGTACCG	GCCGATCACC	GAGTCGGTGC	ACGAACGCCC	GCTGCTGGTG	660
	GTGCCGCCGC	AGATCAACAA	GTTCTACGTC	TTCGACCTGT	CGCCGGACAA	GAGCCTGGCG	720
50	CGCTTCTGCC	TGCGCAACGG	CGTGCAGACC	TTCATCGTCA	GTTGGCGCAA	CCCGACCAAG	780
	TCGCAGCGCG	AATGGGGCCT	GACCACCTAT	ATCGAGGCGC	TCAAGGAGGC	CATCGAGGTA	840
55	GTCCTGTGCA	TCACCGGCAG	CAAGGACCTC	AACCTCCTCG	GCGCCTGCTC	CGGCGGGATC	900

ACCACCGCGA CCCTGGTCGG CCACTACGTG GCCAGCGGCG AGAAGAAGGT CAACGCCTTC 960  
 ACCCAACTGG TCAGCGTGCT CGACTTCGAA CTGAATACCC AGGTCGCGCT GTTCGCCGAC 1020  
 5 GAGAAGACTC TGGAGGCCGC CAAGCGTCGT TCCTACCAGT CCGGCGTGCT GGAGGGCAAG 1080  
 GACATGGCCA AGGTGTTCGC CTGGATGCGC CCCAACGACC TGATCTGGAA CTACTGGGTC 1140  
 AACAACTACC TGCTCGGCAA CCAGCCGCCG GCGTTCGACA TCCTCTACTG GAACAACGAC 1200  
 10 ACCACGCGCC TGCCCGCCGC GCTGCACGGC GAGTTCGTCTG AACTGTTCAA GAGCAACCCG 1260  
 CTGAACCGCC CCGGCGCCCT GGAGGTCTCC GGCACGCCCA TCGACCTGAA GCAGGTGACT 1320  
 15 TCGGACTTCT ACTGTGTCGC CGGTCTGAAC GACCACATCA CCCCCTGGGA GTCGTGCTAC 1380  
 AAGTCGGCCA GGCTGCTGGG TGGCAAGTGC GAGTTCATCC TCTCCAACAG CGGTCACATC 1440  
 CAGAGCATCC TCAACCCACC GGGCAACCCC AAGGCACGCT TCATGACCAA TCCGGAAGTG 1500  
 20 CCCGCCGAGC CCAAGGCCTG GCTGGAACAG GCCGGCAAGC ACGCCGACTC GTGGTGTTG 1560  
 CACTGGCAGC AATGGCTGGC CGAACGCTCC GGCAAGACCC GCAAGGCGCC CGCCAGCCTG 1620  
 25 GGCAACAAGA CCTATCCGGC CGGCGAAGCC GCGCCCGGAA CCTACGTGCA TGAACGA 1677

## (2) INFORMATION FOR SEQ ID NO: 16:

## (i) SEQUENCE CHARACTERISTICS:

- 30 (A) LENGTH: 1680 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

35

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

40 ATGCGTGAAA AGCAGGAATC GGGTAGCGTG CCGGTGCCCC CCGAGTTCAT GAGTGACACAG 60  
 AGCGCCATCG TCGGCCTGCG CGGCAAGGAC CTGCTGACGA CCGTCCGAG CCTGGCTGTC 120  
 45 CACGGCCTGC GCCAGCCGCT GCACAGTGCG CGGCACCTGG TCGCCTTCGG AGGCCAGTTG 180  
 GGCAAGGTGC TGCTGGGCGA CACCCTGCAC CAGCCGAACC CACAGGACGC CCGCTTCCAG 240  
 GATCCATCCT GGCGCCTCAA TCCCTTCTAC CGGCGCACCC TGCAGGCCTA CCTGGCGTGG 300  
 50 CAGAAACAAC TGCTCGCCTG GATCGACGAA AGCAACCTGG ACTGCGACGA TCGCGCCCGC 360  
 GCCCGCTTCC TCGTCGCCTT GCTCTCCGAC GCCGTGGCAC CCAGCAACAG CCTGATCAAT 420  
 55 CCACTGGCGT TAAAGGAACT GTTCAATACC GCGGGGATCA GCCTGCTCAA TGGCGTCCGC 480

CACCTGCTCG AAGACCTGGT GCACAACGGC GGCATGCCCA GCCAGGTGAA CAAGACCGCC 540  
TTCGAGATCG GTCGCAACCT CGCCACCACG CAAGGCGCGG TGGTGTTCGG CAACGAGGTG 600  
5 CTGGAGCTGA TCCAGTACAA GCCGCTGGGC GAGCGCCAGT ACGCCAAGCC CCTGCTGATC 660  
GTGCCGCCGC AGATCAACAA GTACTACATC TTCGACCTGT CGCCGGAAAA GAGCTTCGTC 720  
CAGTACGCCC TGAAGAACAA CCTGCAGGTC TTCGTCATCA GTTGGCGCAA CCCCAGCGCC 780  
10 CAGCACC GCG AATGGGGCCT GAGCACCTAT GTCGAGGCCC TCGACCAGGC CATCGAGGTC 840  
AGCCGCGAGA TCACCGGCAG CCGCAGCGTG AACCTGGCCG GCGCCTGCGC CGGCGGGCTC 900  
15 ACCGTAGCCG CCTTGCTCGG CCACCTGCAG GTGCGCCGGC AACTGCGCAA GGTCAGTAGC 960  
GTCACCTACC TGGTCAGCCT GCTCGACAGC CAGATGGAAA GCCCGGCGAT GCTCTTCGCC 1020  
GACGAGCAGA CCCTGGAGAG CAGCAAGCGC CGCTCCTACC AGCATGGCGT GCTGGACGGG 1080  
20 CGCGACATGG CCAAGGTGTT CGCCTGGATG CGCCCCAACG ACCTGATCTG GAACTACTGG 1140  
GTCAACAACCT ACCTGCTCGG CAGGCAGCCG CCGGCGTTTCG ACATCCTCTA CTGGAACAAC 1200  
25 GACAACACGC GGCTGCCCCG GGC GTTCCAC GGC GAACTGC TCGACCTGTT CAAGCACAAC 1260  
CCGCTGACCC GCCCGGGCGC GCTGGAGGTC AGCGGGACCG CGGTGGACCT GGGCAAGGTG 1320  
GCGATCGACA GCTTCCACGT CGCCGGCATC ACCGACCACA TCACGCCCTG GGACGCGGTG 1380  
30 TATCGCTCGG CCCTCCTGCT GGGCGGCCAG CGCCGCTTCA TCCTGTCCAA CAGCGGGCAC 1440  
ATCCAGAGCA TCCTCAACCC TCCCGGAAAC CCCAAGGCCT GCTACTTCGA GAACGACAAG 1500  
35 CTGAGCAGCG ATCCACGCGC CTGGTACTAC GACGCCAAGC GCGAAGAGGG CAGCTGGTGG 1560  
CCGGTCTGGC TGGGCTGGCT GCAGGAGCGC TCGGGCGAGC TGGGCAACCC TGA CTTC AAC 1620  
CTTGGCAGCG CCGCGCATCC GCCCTCGAA GCGGCCCCGG GCACCTACGT GCATATACGC 1680  
40

## (2) INFORMATION FOR SEQ ID NO: 17:

## (i) SEQUENCE CHARACTERISTICS:

- 45 (A) LENGTH: 1791 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear
- 50

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

55 ATGAGCCAGA AGAACAATAA CGAGCTTCCC AAGCAAGCCG CGGAAAACAC GCTGAACCTG 60

	AATCCGGTGA	TCGGCATCCG	GGGCAAGGAC	CTGCTCACCT	CCGCGCGCAT	GGTCCTGCTC	120
	CAGGCGGTGC	GCCAGCCGCT	GCACAGCGCC	AGGCACGTGG	CGCATTTTCAG	CCTGGAGCTG	180
5	AAGAACGTCC	TGCTCGGCCA	GTCGGAGCTA	CGCCCAGGCG	ATGACGACCG	ACGCTTTTCC	240
	GATCCGGCCT	GGAGCCAGAA	TCCACTGTAC	AAGCGCTACA	TGCAGACCTA	CCTGGCCTGG	300
10	CGCAAGGAGC	TGCACAGCTG	GATCAGCCAC	AGCGACCTGT	CGCCGCAGGA	CATCAGTCGT	360
	GGCCAGTTCG	TCATCAACCT	GCTGACCGAG	GCGATGTCGC	CGACCAACAG	CCTGAGCAAC	420
	CCGGCGGCGG	TCAAGCGCTT	CTTCGAGACC	GGCGGCAAGA	GCCTGCTGGA	CGGCCTCGGC	480
15	CACCTGGCCA	AGGACCTGGT	GAACAACGGC	GGGATGCCGA	GCCAGGTGGA	CATGGACGCC	540
	TTCGAGGTGG	GCAAGAACCT	GGCCACCACC	GAGGGCGCCG	TGGTGTTCGG	CAACGACGTG	600
20	CTGGAACTGA	TCCAGTACCG	GCCGATCACC	GAGTCGGTGC	ACGAACGCCC	GCTGCTGGTG	660
	GTGCCGCCGC	AGATCAACAA	GTTCTACGTC	TTCGACCTGT	CGCCGACAA	GAGCCTGGCG	720
	CGCTTCTGCC	TGCGCAACGG	CGTGCAGACC	TTCATCGTCA	GTTGGCGCAA	CCCGACCAAG	780
25	TCGCAGCGCG	AATGGGGCCT	GACCACCTAT	ATCGAGGCGC	TCAAGGAGGC	CATCGAGGTA	840
	GTCCTGTCGA	TCACCGGCAG	CAAGGACCTC	AACCTCCTCG	GCGCCTGCTC	CGGCGGGATC	900
30	ACCACCGCGA	CCCTGGTCGG	CCACTACGTG	GCCAGCGGCG	AGAAGAAGGT	CAACGCCTTC	960
	ACCCAACTGG	TCAGCGTGCT	CGACTTCGAA	CTGAATACCC	AGGTCGCGCT	GTTGCGCGAC	1020
	GAGAAGACTC	TGGAGGCCGC	CAAGCGTCGT	TCCTACCAGT	CCGGCGTGCT	GGAGGGCAAG	1080
35	GACATGGCCA	AGGTGTTCGC	CTGGATGCGC	CCCAACGACC	TGATCTGGAA	CTACTGGGTC	1140
	AACAACCTACC	TGCTCGGCAA	CCAGCCGCCG	GCGTTCGACA	TCCTCTACTG	GAACAACGAC	1200
40	ACCACGCGCC	TGCCC GCCGC	GCTGCACGGC	GAGTTCGTGC	AACTGTTCAA	GAGCAACCCG	1260
	CTGAACCGCC	CCGGCGCCCT	GGAGGTCTCC	GGCACGCCCA	TCGACCTGAA	GCAGGTGACT	1320
	TGCGACTTCT	ACTGTGTCGC	CGGTCTGAAC	GACCACATCA	CCCCCTGGGA	GTCGTGCTAC	1380
45	AAGTCGGCCA	GGCTGCTGGG	TGGCAAGTGC	GAGTTCATCC	TCTCCAACAG	CGGTCACATC	1440
	CAGAGCATCC	TCAACCCACC	GGGCAACCCC	AAGGCACGCT	TCATGACCAA	TCCGGAAGTG	1500
50	CCCGCCGAGC	CCAAGGCCTG	GCTGGAACAG	GCCGGCAAGC	ACGCCGACTC	GTGGTGTTTG	1560
	CACTGGCAGC	AATGGCTGGC	CGAACGCTCC	GGCAAGACCC	GCAAGGCGCC	CGCCAGCCTG	1620
	GGCAACAAGA	CCTATCCGGC	CGGCGAAGCC	GCGCCCGGAA	CCTACGTGCA	TGAACGATCA	1680
55	AAAGCTTTGG	GCAAAGGTGT	TACCGAGGAA	CAATTCAAAG	AGACCTGGAC	GAGGCCGGGA	1740

GCTGCTGGAA TGGGCGAAGG GACTAGCCTT GTGGTGGCCA AGTCCAGAAT G

1791

## (2) INFORMATION FOR SEQ ID NO: 18:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 597 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

Met Ser Gln Lys Asn Asn Asn Glu Leu Pro Lys Gln Ala Ala Glu Asn  
 1 5 10 15

Thr Leu Asn Leu Asn Pro Val Ile Gly Ile Arg Gly Lys Asp Leu Leu  
 20 25 30

Thr Ser Ala Arg Met Val Leu Leu Gln Ala Val Arg Gln Pro Leu His  
 25 35 40 45

Ser Ala Arg His Val Ala His Phe Ser Leu Glu Leu Lys Asn Val Leu  
 50 55 60

Leu Gly Gln Ser Glu Leu Arg Pro Gly Asp Asp Arg Arg Phe Ser  
 30 65 70 75 80

Asp Pro Ala Trp Ser Gln Asn Pro Leu Tyr Lys Arg Tyr Met Gln Thr  
 35 85 90 95

Tyr Leu Ala Trp Arg Lys Glu Leu His Ser Trp Ile Ser His Ser Asp  
 100 105 110

Leu Ser Pro Gln Asp Ile Ser Arg Gly Gln Phe Val Ile Asn Leu Leu  
 40 115 120 125

Thr Glu Ala Met Ser Pro Thr Asn Ser Leu Ser Asn Pro Ala Ala Val  
 130 135 140

Lys Arg Phe Phe Glu Thr Gly Gly Lys Ser Leu Leu Asp Gly Leu Gly  
 45 145 150 155 160

His Leu Ala Lys Asp Leu Val Asn Asn Gly Gly Met Pro Ser Gln Val  
 165 170 175

Asp Met Asp Ala Phe Glu Val Gly Lys Asn Leu Ala Thr Thr Glu Gly  
 180 185 190

Ala Val Val Phe Arg Asn Asp Val Leu Glu Leu Ile Gln Tyr Arg Pro  
 55 195 200 205

	Ile Thr Glu Ser Val His Glu Arg Pro Leu Leu Val Val Pro Pro Gln	
	210	215 220
5	Ile Asn Lys Phe Tyr Val Phe Asp Leu Ser Pro Asp Lys Ser Leu Ala	
	225	230 235 240
	Arg Phe Cys Leu Arg Asn Gly Val Gln Thr Phe Ile Val Ser Trp Arg	
		245 250 255
10	Asn Pro Thr Lys Ser Gln Arg Glu Trp Gly Leu Thr Thr Tyr Ile Glu	
		260 265 270
	Ala Leu Lys Glu Ala Ile Glu Val Val Leu Ser Ile Thr Gly Ser Lys	
		275 280 285
15	Asp Leu Asn Leu Leu Gly Ala Cys Ser Gly Gly Ile Thr Thr Ala Thr	
		290 295 300
20	Leu Val Gly His Tyr Val Ala Ser Gly Glu Lys Lys Val Asn Ala Phe	
		305 310 315 320
	Thr Gln Leu Val Ser Val Leu Asp Phe Glu Leu Asn Thr Gln Val Ala	
		325 330 335
25	Leu Phe Ala Asp Glu Lys Thr Leu Glu Ala Ala Lys Arg Arg Ser Tyr	
		340 345 350
	Gln Ser Gly Val Leu Glu Gly Lys Asp Met Ala Lys Val Phe Ala Trp	
		355 360 365
30	Met Arg Pro Asn Asp Leu Ile Trp Asn Tyr Trp Val Asn Asn Tyr Leu	
		370 375 380
35	Leu Gly Asn Gln Pro Pro Ala Phe Asp Ile Leu Tyr Trp Asn Asn Asp	
		385 390 395 400
	Thr Thr Arg Leu Pro Ala Ala Leu His Gly Glu Phe Val Glu Leu Phe	
		405 410 415
40	Lys Ser Asn Pro Leu Asn Arg Pro Gly Ala Leu Glu Val Ser Gly Thr	
		420 425 430
	Pro Ile Asp Leu Lys Gln Val Thr Cys Asp Phe Tyr Cys Val Ala Gly	
		435 440 445
45	Leu Asn Asp His Ile Thr Pro Trp Glu Ser Cys Tyr Lys Ser Ala Arg	
		450 455 460
50	Leu Leu Gly Gly Lys Cys Glu Phe Ile Leu Ser Asn Ser Gly His Ile	
		465 470 475 480
	Gln Ser Ile Leu Asn Pro Pro Gly Asn Pro Lys Ala Arg Phe Met Thr	
		485 490 495
55	Asn Pro Glu Leu Pro Ala Glu Pro Lys Ala Trp Leu Glu Gln Ala Gly	
		500 505 510

Lys His Ala Asp Ser Trp Trp Leu His Trp Gln Gln Trp Leu Ala Glu  
 515 520 525  
 5 Arg Ser Gly Lys Thr Arg Lys Ala Pro Ala Ser Leu Gly Asn Lys Thr  
 530 535 540  
 Tyr Pro Ala Gly Glu Ala Ala Pro Gly Thr Tyr Val His Glu Arg Ser  
 545 550 555 560  
 10 Lys Ala Leu Gly Lys Gly Val Thr Glu Glu Gln Phe Lys Glu Thr Trp  
 565 570 575  
 Thr Arg Pro Gly Ala Ala Gly Met Gly Glu Gly Thr Ser Leu Val Val  
 15 580 585 590  
 Ala Lys Ser Arg Met  
 595

20 (2) INFORMATION FOR SEQ ID NO: 19:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1794 base pairs  
 (B) TYPE: nucleic acid  
 25 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

30

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

ATGCGTGAAA AGCAGGAATC GGGTAGCGTG CCGGTGCCCCG CCGAGTTCAT GAGTGCACAG 60  
 35 AGCGCCATCG TCGGCCTGCG CGGCAAGGAC CTGCTGACGA CGGTCCGCAG CCTGGCTGTC 120  
 CACGGCCTGC GCCAGCCGCT GCACAGTGCG CGGCACCTGG TCGCCTTCGG AGGCCAGTTG 180  
 40 GGCAAGGTGC TGCTGGGCGA CACCCTGCAC CAGCCGAACC CACAGGACGC CCGCTTCCAG 240  
 GATCCATCCT GGCGCCTCAA TCCCTTCTAC CGGCGCACCC TGCAGGCCTA CCTGGCGTGG 300  
 CAGAAACAAC TGCTCGCCTG GATCGACGAA AGCAACCTGG ACTGCGACGA TCGCGCCCCG 360  
 45 GCGCGCTTCC TCGTCGCCTT GCTCTCCGAC GCCGTGGCAC CCAGCAACAG CCTGATCAAT 420  
 CCACTGGCGT TAAAGGAACT GTTCAATACC GGCGGGATCA GCCTGCTCAA TGGCGTCCGC 480  
 50 CACCTGCTCG AAGACCTGGT GCACAACGGC GGCATGCCCA GCCAGGTGAA CAAGACCGCC 540  
 TTCGAGATCG GTCGCAACCT CGCCACCACG CAAGGCGCGG TGGTGTTCGG CAACGAGGTG 600  
 CTGGAGCTGA TCCAGTACAA GCCGCTGGGC GAGCGCCAGT ACGCCAAGCC CCTGCTGATC 660  
 55 GTGCCGCCGC AGATCAACAA GTACTACATC TTCGACCTGT CGCCGGAAAA GAGCTTCGTC 720



CAGTACGCCC TGAAGAACAA CCTGCAGGTC TTCGTCATCA GTTGGCGCAA CCCCAGCGCC 780  
 CAGCACCGCG AATGGGGCCT GAGCACCTAT GTCGAGGCCC TCGACCAGGC CATCGAGGTC 840  
 5 AGCCGCGAGA TCACCGGCAG CCGCAGCGTG AACCTGGCCG GCGCCTGCGC CGGCGGGCTC 900  
 ACCGTAGCCG CCTTGCTCGG CCACCTGCAG GTGCGCCGGC AACTGCGCAA GGTCAGTAGC 960  
 10 GTCACCTACC TGGTCAGCCT GCTCGACAGC CAGATGGAAA GCCC GGCGAT GCTCTTCGCC 1020  
 GACGAGCAGA CCCTGGAGAG CAGCAAGCGC CGCTCCTACC AGCATGGCGT GCTGGACGGG 1080  
 CGCGACATGG CCAAGGTGTT CGCCTGGATG CGCCCCAACG ACCTGATCTG GAACTACTGG 1140  
 15 GTCAACAACCT ACCTGCTCGG CAGGCAGCCG CCGGCGTTCTG ACATCCTCTA CTGGAACAAC 1200  
 GACAACACGC GGCTGCCCCG GCGGTTCCAC GCGGAAGTGC TCGACCTGTT CAAGCACAAC 1260  
 20 CCGCTGACCC GCCC GGCGC GCTGGAGGTC AGCGGGACCG CCGTGGACCT GGGCAAGGTG 1320  
 GCGATCGACA GCTTCCACGT CGCCGGCATC ACCGACCACA TCACGCCCTG GGACGCGGTG 1380  
 TATCGCTCGG CCCTCCTGCT GGGCGGCCAG CGCCGCTTCA TCCTGTCCAA CAGCGGGCAC 1440  
 25 ATCCAGAGCA TCCTCAACCC TCCCGGAAAC CCCAAGGCCT GCTACTTCGA GAACGACAAG 1500  
 CTGAGCAGCG ATCCACGCGC CTGGTACTAC GACGCCAAGC GCGAAGAGGG CAGCTGGTGG 1560  
 30 CCGGTCTGGC TGGGCTGGCT GCAGGAGCGC TCGGGCGAGC TGGGCAACCC TGACTTCAAC 1620  
 CTTGGCAGCG CCGCGCATCC GCCCTCGAA GCGGCCCCGG GCACCTACGT GCATATACGC 1680  
 TCAAAAGCTT TGGGCAAAGG TGTTACCGAG GAACAATTCA AAGAGACCTG GACGAGGCCG 1740  
 35 GGAGCTGCTG GAATGGGCGA AGGGACTAGC CTTGTGGTGG CCAAGTCCAG AATG 1794

## (2) INFORMATION FOR SEQ ID NO: 20:

- 40 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 598 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS:  
 45 (D) TOPOLOGY: linear

## 50 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

Met Arg Glu Lys Gln Glu Ser Gly Ser Val Pro Val Pro Ala Glu Phe  
 1 5 10 15  
 55 Met Ser Ala Gln Ser Ala Ile Val Gly Leu Arg Gly Lys Asp Leu Leu  
 20 25 30

Thr Thr Val Arg Ser Leu Ala Val His Gly Leu Arg Gln Pro Leu His  
 35 40 45  
 5 Ser Ala Arg His Leu Val Ala Phe Gly Gly Gln Leu Gly Lys Val Leu  
 50 55 60  
 Leu Gly Asp Thr Leu His Gln Pro Asn Pro Gln Asp Ala Arg Phe Gln  
 65 70 75 80  
 10 Asp Pro Ser Trp Arg Leu Asn Pro Phe Tyr Arg Arg Thr Leu Gln Ala  
 85 90 95  
 Tyr Leu Ala Trp Gln Lys Gln Leu Leu Ala Trp Ile Asp Glu Ser Asn  
 100 105 110  
 15 Leu Asp Cys Asp Asp Arg Ala Arg Ala Arg Phe Leu Val Ala Leu Leu  
 115 120 125  
 Ser Asp Ala Val Ala Pro Ser Asn Ser Leu Ile Asn Pro Leu Ala Leu  
 130 135 140  
 Lys Glu Leu Phe Asn Thr Gly Gly Ile Ser Leu Leu Asn Gly Val Arg  
 145 150 155 160  
 25 His Leu Leu Glu Asp Leu Val His Asn Gly Gly Met Pro Ser Gln Val  
 165 170 175  
 Asn Lys Thr Ala Phe Glu Ile Gly Arg Asn Leu Ala Thr Thr Gln Gly  
 180 185 190  
 30 Ala Val Val Phe Arg Asn Glu Val Leu Glu Leu Ile Gln Tyr Lys Pro  
 195 200 205  
 Leu Gly Glu Arg Gln Tyr Ala Lys Pro Leu Leu Ile Val Pro Pro Gln  
 210 215 220  
 Ile Asn Lys Tyr Tyr Ile Phe Asp Leu Ser Pro Glu Lys Ser Phe Val  
 225 230 235 240  
 40 Gln Tyr Ala Leu Lys Asn Asn Leu Gln Val Phe Val Ile Ser Trp Arg  
 245 250 255  
 Asn Pro Asp Ala Gln His Arg Glu Trp Gly Leu Ser Thr Tyr Val Glu  
 260 265 270  
 45 Ala Leu Asp Gln Ala Ile Glu Val Ser Arg Glu Ile Thr Gly Ser Arg  
 275 280 285  
 Ser Val Asn Leu Ala Gly Ala Cys Ala Gly Gly Leu Thr Val Ala Ala  
 290 295 300  
 50 Leu Leu Gly His Leu Gln Val Arg Arg Gln Leu Arg Lys Val Ser Ser  
 305 310 315 320  
 55 Val Thr Tyr Leu Val Ser Leu Leu Asp Ser Gln Met Glu Ser Pro Ala

	325	330	335
	Met Leu Phe Ala Asp Glu Gln Thr	Leu Glu Ser Ser Lys Arg Arg Ser	
	340	345	350
5	Tyr Gln His Gly Val Leu Asp Gly Arg Asp Met Ala Lys Val Phe Ala		
	355	360	365
10	Trp Met Arg Pro Asn Asp Leu Ile Trp Asn Tyr Trp Val Asn Asn Tyr		
	370	375	380
	Leu Leu Gly Arg Gln Pro Pro Ala Phe Asp Ile Leu Tyr Trp Asn Asn		
	385	390	395
15	Asp Asn Thr Arg Leu Pro Ala Ala Phe His Gly Glu Leu Leu Asp Leu		
	405	410	415
	Phe Lys His Asn Pro Leu Thr Arg Pro Gly Ala Leu Glu Val Ser Gly		
	420	425	430
20	Thr Ala Val Asp Leu Gly Lys Val Ala Ile Asp Ser Phe His Val Ala		
	435	440	445
	Gly Ile Thr Asp His Ile Thr Pro Trp Asp Ala Val Tyr Arg Ser Ala		
25	450	455	460
	Leu Leu Leu Gly Gly Gln Arg Arg Phe Ile Leu Ser Asn Ser Gly His		
	465	470	475
30	Ile Gln Ser Ile Leu Asn Pro Pro Gly Asn Pro Lys Ala Cys Tyr Phe		
	485	490	495
	Glu Asn Asp Lys Leu Ser Ser Asp Pro Arg Ala Trp Tyr Tyr Asp Ala		
	500	505	510
35	Lys Arg Glu Glu Gly Ser Trp Trp Pro Val Trp Leu Gly Trp Leu Gln		
	515	520	525
	Glu Arg Ser Gly Glu Leu Gly Asn Pro Asp Phe Asn Leu Gly Ser Ala		
40	530	535	540
	Ala His Pro Pro Leu Glu Ala Ala Pro Gly Thr Tyr Val His Ile Arg		
	545	550	555
45	Ser Lys Ala Leu Gly Lys Gly Val Thr Glu Glu Gln Phe Lys Glu Thr		
	565	570	575
	Trp Thr Arg Pro Gly Ala Ala Gly Met Gly Glu Gly Thr Ser Leu Val		
	580	585	590
50	Val Ala Lys Ser Arg Met		
	595		

(2) INFORMATION FOR SEQ ID NO: 21:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 2737 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

5

10 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:

GAATTCATGT CTCCAGTTGA TTTTAAAGAT AAAGTTGTGA TCATTACCGG TGCCGGTGGT 60  
GGTTTGGGTA AATACTACTC CCTCGAATTT GCCAAGTTGG GCGCCAAAGT CGTCGTTAAC 120  
15 GACTTGGGTG GTGCCTTGAA CGGTCAAGGT GGAAACTCCA AGGCCGCCGA CGTTGTCGTT 180  
GACGAAATTG TCAAGAACGG TGGTGTGGCC GTTGCCGATT ACAACAACGT CTTGGACGGT 240  
20 GACAAGATTG TCGAAACCGC CGTCAAGAAC TTTGGTACTG TCCACGTTAT CATCAACAAT 300  
GCCGGTATCT TGAGAGATGC CTCCATGAAG AAGATGACTG AAAAAGACTA CAAATTGGTC 360  
ATTGACGTGC ACTTGAACGG TGCCTTTGCC GTCACCAAGG CTGCTTGGCC ATACTTCCAA 420  
25 AAGCAAAAAT ACGGTAGAAT TGTCAACACA TCCTCCCCAG CTGGTTTGTA CGGTAACTTT 480  
GGTCAAGCCA ACTACGCCTC CGCCAAGTCT GCTTTGTTGG GATTCGCTGA AACCTTGGCC 540  
30 AAGGAAGGTG CCAAATACAA CATCAAGGCC AACGCCATTG CTCCGTTGGC CAGATCAAGA 600  
ATGACTGAAT CTATCTTGCC ACCTCCAATG TTGGAAAAAT TGGGCCCTGA AAAGGTTGCC 660  
CCATTGGTCT TGTATTTGTC GTCAGCTGAA AACGAATTGA CTGGTCAATT CTTTGAAGTT 720  
35 GCTGCTGGCT TTTACGCTCA GATCAGATGG GAAAGATCCG GTGGTGCTTT GTTCAAGCCA 780  
GATCAATCCT TCACCGCTGA GGTGTGTGCT AAGAGATTCT CTGAAATCCT TGATTATGAC 840  
40 GACTCTAGGA AGCCAGAATA CTTGAAGAAC CAATACCCAT TCATGTTGAA CGACTACGCC 900  
ACTTTGACCA ACGAAGCTAG AAAGTTGCCA GCTAACGATG CTTCTGGTGC TCCAAC TGTC 960  
TCCTTGAAGG ACAAGGTTGT TTTGATCACC GGTGCCGGTG CTGGTTTGGG TAAAGAATAC 1020  
45 GCCAAGTGGT TCGCCAAGTA CGGTGCCAAG GTTGTGTGTTA ACGACTTCAA GGATGCTACC 1080  
AAGACCGTTG ACGAAATCAA AGCCGCTGGT GGTGAAGCTT GGCCAGATCA ACACGATGTT 1140  
50 GCCAAGGACT CCGAAGCTAT CATCAAGAAT GTCATTGACA AGTACGGTAC CATTGATATC 1200  
TTGGTCAACA ACGCCGGTAT CTTGAGAGAC AGATCCTTTG CCAAGATGTC CAAGCAAGAA 1260  
TGGGACTCTG TCCAACAAGT CCACCTTGATT GGTACTTTCA ACTTGAGCAG ATTGGCATGG 1320  
55 CCATACTTTG TTGAAAAACA ATTTGGTAGA ATCATCAACA TTACCTCCAC CAGTGGTATC 1380

TACGGTAACT TTGGTCAAGC CAACTACTCG TCTTCTAAGG CTGGTATCTT GGGTTTGTCC 1440  
 AAGACCATGG CCATTGAAGG TGCTAAGAAT AACATTAAGG TCAACATTGT TGCTCCACAC 1500  
 5 GCTGAAACTG CCATGACCTT GACCATCTTC AGAGAACAAG ACAAGAACTT GTACCACGCT 1560  
 GACCAAGTTG CTCCATTGTT GGTCTACTTG GGTACTGACG ATGTCCCAGT CACCGGTGAA 1620  
 10 ACTTCCGAAA TCGGTGGTGG TTGGATCGGT AACACCAGAT GGCAAAGAGC CAAGGGTGCT 1680  
 GTCTCCCACG ACGAACACAC CACTGTTGAA TTCATCAAGG AGCACTTGAA CGAAATCACT 1740  
 GACTTCACCA CTGACACTGA AAATCCAAAA TCTACCACCG AATCCTCCAT GGCTATCTTG 1800  
 15 TCTGCCGTTG GTGGTGATGA CGATGATGAT GACGAAGACG AAGAAGAAGA CGAAGGTGAT 1860  
 GAAGAAGAAG ACGAAGAAGA CGAAGAAGAA GACGATCCAG TCTGGAGATT CGACGACAGA 1920  
 20 GATGTTATCT TGTACAACAT TGCCCTTGGT GCCACCACCA AGCAATTGAA GTACGTCTAC 1980  
 GAAAACGACT CTGACTTCCA AGTCATTCCA ACCTTTGGTC ACTTGATCAC CTTCAACTCT 2040  
 GGTAAGTCAC AAAACTCCTT TGCCAAGTTG TTGCGTAACT TCAACCCAAT GTTGTGTTG 2100  
 25 CACGGTGAAC ACTACTTGAA GGTGCACAGC TGGCCACCAC CAACCGAAGG TGAAATCAAG 2160  
 ACCACTTTCG AACCAATTGC CACTACTCCA AAGGGTACCA ACGTTGTTAT TGTTCCACGGT 2220  
 30 TCCAAATCTG TTGACAACAA GTCTGGTGAA TTGATTTACT CCAACGAAGC CACTTACTTC 2280  
 ATCAGAACT GTCAAGCCGA CAACAAGGTC TACGCTGACC GTCCAGCATT CGCCACCAAC 2340  
 CAATTCCTGG CACCAAAGAG AGCCCCAGAC TACCAAGTTG ACGTTCCAGT CAGTGAAGAC 2400  
 35 TTGGCTGCTT TGTACCGTTT GTCTGGTGAC AGAAACCCAT TGCACATTGA TCCAAACTTT 2460  
 GCTAAAGGTG CCAAGTTCCC TAAGCCAATC TTACACGGTA TGTGCACTTA TGGTTTGAGT 2520  
 40 GCTAAGGCTT TGATTGACAA GTTTGGTATG TTCAACGAAA TCAAGGCCAG ATTCACCGGT 2580  
 ATTGTCTTCC CAGGTGAAAC CTTGAGAGTC TTGGCATGGA AGGAAAGCGA TGACACTATT 2640  
 GTCTTCCAAA CTCATGTTGT TGATAGAGGT ACTATTGCCA TTAACAACGC TGCTATTAAG 2700  
 45 TTAGTCGGTG ACAAAGCAAA GATCTAATGA AGGATCC 2737

(2) INFORMATION FOR SEQ ID NO: 22:

- 50 (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 906 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS:
  - (D) TOPOLOGY: linear
- 55

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:

5 Met Ser Pro Val Asp Phe Lys Asp Lys Val Val Ile Ile Thr Gly Ala  
 1 5 10 15  
 10 Gly Gly Gly Leu Gly Lys Tyr Tyr Ser Leu Glu Phe Ala Lys Leu Gly  
 20 25 30  
 Ala Lys Val Val Val Asn Asp Leu Gly Gly Ala Leu Asn Gly Gln Gly  
 35 40 45  
 15 Gly Asn Ser Lys Ala Ala Asp Val Val Val Asp Glu Ile Val Lys Asn  
 50 55 60  
 Gly Gly Val Ala Val Ala Asp Tyr Asn Asn Val Leu Asp Gly Asp Lys  
 65 70 75 80  
 20 Ile Val Glu Thr Ala Val Lys Asn Phe Gly Thr Val His Val Ile Ile  
 85 90 95  
 Asn Asn Ala Gly Ile Leu Arg Asp Ala Ser Met Lys Lys Met Thr Glu  
 100 105 110  
 25 Lys Asp Tyr Lys Leu Val Ile Asp Val His Leu Asn Gly Ala Phe Ala  
 115 120 125  
 30 Val Thr Lys Ala Ala Trp Pro Tyr Phe Gln Lys Gln Lys Tyr Gly Arg  
 130 135 140  
 Ile Val Asn Thr Ser Ser Pro Ala Gly Leu Tyr Gly Asn Phe Gly Gln  
 145 150 155 160  
 35 Ala Asn Tyr Ala Ser Ala Lys Ser Ala Leu Leu Gly Phe Ala Glu Thr  
 165 170 175  
 40 Leu Ala Lys Glu Gly Ala Lys Tyr Asn Ile Lys Ala Asn Ala Ile Ala  
 180 185 190  
 Pro Leu Ala Arg Ser Arg Met Thr Glu Ser Ile Leu Pro Pro Pro Met  
 195 200 205  
 45 Leu Glu Lys Leu Gly Pro Glu Lys Val Ala Pro Leu Val Leu Tyr Leu  
 210 215 220  
 Ser Ser Ala Glu Asn Glu Leu Thr Gly Gln Phe Phe Glu Val Ala Ala  
 225 230 235 240  
 50 Gly Phe Tyr Ala Gln Ile Arg Trp Glu Arg Ser Gly Gly Val Leu Phe  
 245 250 255  
 55 Lys Pro Asp Gln Ser Phe Thr Ala Glu Val Val Ala Lys Arg Phe Ser  
 260 265 270

	Glu	Ile	Leu	Asp	Tyr	Asp	Asp	Ser	Arg	Lys	Pro	Glu	Tyr	Leu	Lys	Asn	
			275					280					285				
5	Gln	Tyr	Pro	Phe	Met	Leu	Asn	Asp	Tyr	Ala	Thr	Leu	Thr	Asn	Glu	Ala	
		290					295					300					
	Arg	Lys	Leu	Pro	Ala	Asn	Asp	Ala	Ser	Gly	Ala	Pro	Thr	Val	Ser	Leu	
	305					310					315					320	
10	Lys	Asp	Lys	Val	Val	Leu	Ile	Thr	Gly	Ala	Gly	Ala	Gly	Leu	Gly	Lys	
					325					330					335		
	Glu	Tyr	Ala	Lys	Trp	Phe	Ala	Lys	Tyr	Gly	Ala	Lys	Val	Val	Val	Asn	
				340					345					350			
15	Asp	Phe	Lys	Asp	Ala	Thr	Lys	Thr	Val	Asp	Glu	Ile	Lys	Ala	Ala	Gly	
			355					360					365				
	Gly	Glu	Ala	Trp	Pro	Asp	Gln	His	Asp	Val	Ala	Lys	Asp	Ser	Glu	Ala	
20		370					375					380					
	Ile	Ile	Lys	Asn	Val	Ile	Asp	Lys	Tyr	Gly	Thr	Ile	Asp	Ile	Leu	Val	
	385					390					395					400	
25	Asn	Asn	Ala	Gly	Ile	Leu	Arg	Asp	Arg	Ser	Phe	Ala	Lys	Met	Ser	Lys	
					405					410					415		
	Gln	Glu	Trp	Asp	Ser	Val	Gln	Gln	Val	His	Leu	Ile	Gly	Thr	Phe	Asn	
				420					425					430			
30	Leu	Ser	Arg	Leu	Ala	Trp	Pro	Tyr	Phe	Val	Glu	Lys	Gln	Phe	Gly	Arg	
			435					440					445				
	Ile	Ile	Asn	Ile	Thr	Ser	Thr	Ser	Gly	Ile	Tyr	Gly	Asn	Phe	Gly	Gln	
35		450					455					460					
	Ala	Asn	Tyr	Ser	Ser	Ser	Lys	Ala	Gly	Ile	Leu	Gly	Leu	Ser	Lys	Thr	
	465					470					475					480	
40	Met	Ala	Ile	Glu	Gly	Ala	Lys	Asn	Asn	Ile	Lys	Val	Asn	Ile	Val	Ala	
					485					490					495		
	Pro	His	Ala	Glu	Thr	Ala	Met	Thr	Leu	Thr	Ile	Phe	Arg	Glu	Gln	Asp	
				500					505					510			
45	Lys	Asn	Leu	Tyr	His	Ala	Asp	Gln	Val	Ala	Pro	Leu	Leu	Val	Tyr	Leu	
			515					520					525				
	Gly	Thr	Asp	Asp	Val	Pro	Val	Thr	Gly	Glu	Thr	Ser	Glu	Ile	Gly	Gly	
50		530					535					540					
	Gly	Trp	Ile	Gly	Asn	Thr	Arg	Trp	Gln	Arg	Ala	Lys	Gly	Ala	Val	Ser	
	545					550					555					560	
55	His	Asp	Glu	His	Thr	Thr	Val	Glu	Phe	Ile	Lys	Glu	His	Leu	Asn	Glu	
					565					570					575		

	Ile Thr Asp Phe Thr Thr Asp Thr Glu Asn Pro Lys Ser Thr Thr Glu	580	585	590
5	Ser Ser Met Ala Ile Leu Ser Ala Val Gly Gly Asp Asp Asp Asp Asp	595	600	605
	Asp Glu Asp Glu Glu Glu Asp Glu Gly Asp Glu Glu Glu Asp Glu Glu	610	615	620
10	Asp Glu Glu Glu Asp Asp Pro Val Trp Arg Phe Asp Asp Arg Asp Val	625	630	635
	Ile Leu Tyr Asn Ile Ala Leu Gly Ala Thr Thr Lys Gln Leu Lys Tyr	645	650	655
15	Val Tyr Glu Asn Asp Ser Asp Phe Gln Val Ile Pro Thr Phe Gly His	660	665	670
20	Leu Ile Thr Phe Asn Ser Gly Lys Ser Gln Asn Ser Phe Ala Lys Leu	675	680	685
	Leu Arg Asn Phe Asn Pro Met Leu Leu Leu His Gly Glu His Tyr Leu	690	695	700
25	Lys Val His Ser Trp Pro Pro Pro Thr Glu Gly Glu Ile Lys Thr Thr	705	710	715
	Phe Glu Pro Ile Ala Thr Thr Pro Lys Gly Thr Asn Val Val Ile Val	725	730	735
30	His Gly Ser Lys Ser Val Asp Asn Lys Ser Gly Glu Leu Ile Tyr Ser	740	745	750
35	Asn Glu Ala Thr Tyr Phe Ile Arg Asn Cys Gln Ala Asp Asn Lys Val	755	760	765
	Tyr Ala Asp Arg Pro Ala Phe Ala Thr Asn Gln Phe Leu Ala Pro Lys	770	775	780
40	Arg Ala Pro Asp Tyr Gln Val Asp Val Pro Val Ser Glu Asp Leu Ala	785	790	795
	Ala Leu Tyr Arg Leu Ser Gly Asp Arg Asn Pro Leu His Ile Asp Pro	805	810	815
45	Asn Phe Ala Lys Gly Ala Lys Phe Pro Lys Pro Ile Leu His Gly Met	820	825	830
50	Cys Thr Tyr Gly Leu Ser Ala Lys Ala Leu Ile Asp Lys Phe Gly Met	835	840	845
	Phe Asn Glu Ile Lys Ala Arg Phe Thr Gly Ile Val Phe Pro Gly Glu	850	855	860
55	Thr Leu Arg Val Leu Ala Trp Lys Glu Ser Asp Asp Thr Ile Val Phe			



865	870	875	880
Gln Thr His Val Val Asp Arg Gly Thr Ile Ala Ile Asn Asn Ala Ala			
885	890	895	
Ile Lys Leu Val Gly Asp Lys Ala Lys Ile			
900	905		

## (2) INFORMATION FOR SEQ ID NO: 23:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2737 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23:

GGATCCATGT CTCCAGTTGA TTTTAAAGAT AAAGTTGTGA TCATTACCGG TGCCGGTGGT	60
GGTTTGGGTA AATACTACTC CCTCGAATTT GCCAAGTTGG GCGCCAAAGT CGTCGTTAAC	120
GACTTGGGTG GTGCCTTGAA CGGTCAAGGT GGAAACTCCA AGGCCGCCGA CGTTGTCGTT	180
GACGAAATTG TCAAGAACGG TGGTGTGTC GTTGCCGATT ACAACAACGT CTTGGACGGT	240
GACAAGATTG TCGAAACCGC CGTCAAGAAC TTTGGTACTG TCCACGTTAT CATCAACAAT	300
GCCGGTATCT TGAGAGATGC CTCCATGAAG AAGATGACTG AAAAAGACTA CAAATTGGTC	360
ATTGACGTGC ACTTGAACGG TGCCTTTGCC GTCACCAAGG CTGCTTGGCC ATACTTCCAA	420
AAGCAAAAT ACGGTAGAAT TGTCAACACA TCCTCCCCAG CTGGTTTGTA CGGTAACTTT	480
GGTCAAGCCA ACTACGCCTC CGCCAAGTCT GCTTTGTTGG GATTCGCTGA AACCTTGGCC	540
AAGGAAGGTG CCAAATACAA CATCAAGGCC AACGCCATTG CTCCGTTGGC CAGATCAAGA	600
ATGACTGAAT CTATCTTGCC ACCTCCAATG TTGGAAAAAT TGGGCCCTGA AAAGGTTGCC	660
CCATTGGTCT TGTATTTGTC GTCAGCTGAA AACGAATTGA CTGGTCAATT CTTTGAAGTT	720
GCTGCTGGCT TTTACGCTCA GATCAGATGG GAAAGATCCG GTGGTGTCTT GTTCAAGCCA	780
GATCAATCCT TCACCGCTGA GGTGTGTGCT AAGAGATTCT CTGAAATCCT TGATTATGAC	840
GACTCTAGGA AGCCAGAATA CTTGAAGAAC CAATACCCAT TCATGTTGAA CGACTACGCC	900
ACTTTGACCA ACGAAGCTAG AAAGTTGCCA GCTAACGATG CTTCTGGTGC TCCAACGTGC	960
TCCTTGAAGG ACAAGGTTGT TTTGATCACC GGTGCCGGTG CTGGTTTGGG TAAAGAATAC	1020

	GCCAAAGTGGT	TCGCCAAGTA	CGGTGCCAAG	GTTGTTGTTA	ACGACTTCAA	GGATGCTACC	1080
	AAGACCGTTG	ACGAAATCAA	AGCCGCTGGT	GGTGAAGCTT	GGCCAGATCA	ACACGATGTT	1140
5	GCCAAAGGACT	CCGAAGCTAT	CATCAAGAAT	GTCATTGACA	AGTACGGTAC	CATTGATATC	1200
	TTGGTCAACA	ACGCCGGTAT	CTTGAGAGAC	AGATCCTTTG	CCAAGATGTC	CAAGCAAGAA	1260
	TGGGACTCTG	TCCAACAAGT	CCACTTGATT	GGTACTTTCA	ACTTGAGCAG	ATTGGCATGG	1320
10	CCATACTTTG	TTGAAAAACA	ATTTGGTAGA	ATCATCAACA	TTACCTCCAC	CAGTGGTATC	1380
	TACGGTAACT	TTGGTCAAGC	CAACTACTCG	TCTTCTAAGG	CTGGTATCTT	GGGTTTGTCC	1440
15	AAGACCATGG	CCATTGAAGG	TGCTAAGAAT	AACATTAAGG	TCAACATTGT	TGCTCCACAC	1500
	GCTGAAACTG	CCATGACCTT	GACCATCTTC	AGAGAACAAG	ACAAGAACTT	GTACCACGCT	1560
	GACCAAGTTG	CTCCATTGTT	GGTCTACTTG	GGTACTGACG	ATGTCCCAGT	CACCGGTGAA	1620
20	ACTTCCGAAA	TCGGTGGTGG	TTGGATCGGT	AACACCAGAT	GGCAAAGAGC	CAAGGGTGCT	1680
	GTCTCCCACG	ACGAACACAC	CACTGTTGAA	TTCATCAAGG	AGCACTTGAA	CGAAATCACT	1740
25	GACTTCACCA	CTGACACTGA	AAATCCAAAA	TCTACCACCG	AATCCTCCAT	GGCTATCTTG	1800
	TCTGCCGTTG	GTGGTGATGA	CGATGATGAT	GACGAAGACG	AAGAAGAAGA	CGAAGGTGAT	1860
	GAAGAAGAAG	ACGAAGAAGA	CGAAGAAGAA	GACGATCCAG	TCTGGAGATT	CGACGACAGA	1920
30	GATGTTATCT	TGTACAACAT	TGCCCTTGGT	GCCACCACCA	AGCAATTGAA	GTACGTCTAC	1980
	GAAAACGACT	CTGACTTCCA	AGTCATTCCA	ACCTTTGGTC	ACTTGATCAC	CTTCAACTCT	2040
35	GGTAAGTCAC	AAAACCTCCTT	TGCCAAGTTG	TTGCGTAACT	TCAACCCAAT	GTTGTTGTTG	2100
	CACGGTGAAC	ACTACTTGAA	GGTGCACAGC	TGGCCACCAC	CAACCGAAGG	TGAAATCAAG	2160
	ACCACTTTCG	AACCAATTGC	CACTACTCCA	AAGGGTACCA	ACGTTGTTAT	TGTTACCGGT	2220
40	TCCAAATCTG	TTGACAACAA	GTCTGGTGAA	TTGATTTACT	CCAACGAAGC	CACTTACTTC	2280
	ATCAGAAACT	GTCAAGCCGA	CAACAAGGTC	TACGCTGACC	GTCCAGCATT	CGCCACCAAC	2340
45	CAATTCTTGG	CACCAAAGAG	AGCCCCAGAC	TACCAAGTTG	ACGTTCCAGT	CAGTGAAGAC	2400
	TTGGCTGCTT	TGTACCGTTT	GTCTGGTGAC	AGAAACCCAT	TGCACATTGA	TCCAAACTTT	2460
	GCTAAAGGTG	CCAAGTTCCC	TAAGCCAATC	TTACACGGTA	TGTGCACTTA	TGGTTTGAGT	2520
50	GCTAAGGCTT	TGATTGACAA	GTTTGGTATG	TTCAACGAAA	TCAAGGCCAG	ATTCACCGGT	2580
	ATTGTCTTCC	CAGGTGAAAC	CTTGAGAGTC	TTGGCATGGA	AGGAAAGCGA	TGACACTATT	2640
55	GTCTTCCAAA	CTCATGTTGT	TGATAGAGGT	ACTATTGCCA	TTAACAACGC	TGCTATTAAG	2700

TTAGTCGGTG ACAAATCCAA GTTGTAAATGA AGGATCC

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## (2) INFORMATION FOR SEQ ID NO: 24:

- 5 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 906 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS:  
 (D) TOPOLOGY: linear

## 15 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:

Met Ser Pro Val Asp Phe Lys Asp Lys Val Val Ile Ile Thr Gly Ala  
 1 5 10 15

Gly Gly Gly Leu Gly Lys Tyr Tyr Ser Leu Glu Phe Ala Lys Leu Gly  
 20 25 30

Ala Lys Val Val Val Asn Asp Leu Gly Gly Ala Leu Asn Gly Gln Gly  
 35 40 45

Gly Asn Ser Lys Ala Ala Asp Val Val Val Asp Glu Ile Val Lys Asn  
 50 55 60

Gly Gly Val Ala Val Ala Asp Tyr Asn Asn Val Leu Asp Gly Asp Lys  
 65 70 75 80

Ile Val Glu Thr Ala Val Lys Asn Phe Gly Thr Val His Val Ile Ile  
 85 90 95

Asn Asn Ala Gly Ile Leu Arg Asp Ala Ser Met Lys Lys Met Thr Glu  
 100 105 110

Lys Asp Tyr Lys Leu Val Ile Asp Val His Leu Asn Gly Ala Phe Ala  
 115 120 125

Val Thr Lys Ala Ala Trp Pro Tyr Phe Gln Lys Gln Lys Tyr Gly Arg  
 130 135 140

Ile Val Asn Thr Ser Ser Pro Ala Gly Leu Tyr Gly Asn Phe Gly Gln  
 145 150 155 160

Ala Asn Tyr Ala Ser Ala Lys Ser Ala Leu Leu Gly Phe Ala Glu Thr  
 165 170 175

Leu Ala Lys Glu Gly Ala Lys Tyr Asn Ile Lys Ala Asn Ala Ile Ala  
 180 185 190

Pro Leu Ala Arg Ser Arg Met Thr Glu Ser Ile Leu Pro Pro Pro Met  
 195 200 205

Leu Glu Lys Leu Gly Pro Glu Lys Val Ala Pro Leu Val Leu Tyr Leu

	210	215	220
	Ser Ser Ala Glu Asn Glu Leu Thr Gly Gln Phe Phe Glu Val Ala Ala		
	225	230	235 240
5	Gly Phe Tyr Ala Gln Ile Arg Trp Glu Arg Ser Gly Gly Val Leu Phe		
	245	250	255
10	Lys Pro Asp Gln Ser Phe Thr Ala Glu Val Val Ala Lys Arg Phe Ser		
	260	265	270
	Glu Ile Leu Asp Tyr Asp Asp Ser Arg Lys Pro Glu Tyr Leu Lys Asn		
	275	280	285
15	Gln Tyr Pro Phe Met Leu Asn Asp Tyr Ala Thr Leu Thr Asn Glu Ala		
	290	295	300
	Arg Lys Leu Pro Ala Asn Asp Ala Ser Gly Ala Pro Thr Val Ser Leu		
	305	310	315 320
20	Lys Asp Lys Val Val Leu Ile Thr Gly Ala Gly Ala Gly Leu Gly Lys		
	325	330	335
	Glu Tyr Ala Lys Trp Phe Ala Lys Tyr Gly Ala Lys Val Val Val Asn		
25	340	345	350
	Asp Phe Lys Asp Ala Thr Lys Thr Val Asp Glu Ile Lys Ala Ala Gly		
	355	360	365
30	Gly Glu Ala Trp Pro Asp Gln His Asp Val Ala Lys Asp Ser Glu Ala		
	370	375	380
	Ile Ile Lys Asn Val Ile Asp Lys Tyr Gly Thr Ile Asp Ile Leu Val		
	385	390	395 400
35	Asn Asn Ala Gly Ile Leu Arg Asp Arg Ser Phe Ala Lys Met Ser Lys		
	405	410	415
	Gln Glu Trp Asp Ser Val Gln Gln Val His Leu Ile Gly Thr Phe Asn		
40	420	425	430
	Leu Ser Arg Leu Ala Trp Pro Tyr Phe Val Glu Lys Gln Phe Gly Arg		
	435	440	445
45	Ile Ile Asn Ile Thr Ser Thr Ser Gly Ile Tyr Gly Asn Phe Gly Gln		
	450	455	460
	Ala Asn Tyr Ser Ser Ser Lys Ala Gly Ile Leu Gly Leu Ser Lys Thr		
	465	470	475 480
50	Met Ala Ile Glu Gly Ala Lys Asn Asn Ile Lys Val Asn Ile Val Ala		
	485	490	495
	Pro His Ala Glu Thr Ala Met Thr Leu Thr Ile Phe Arg Glu Gln Asp		
55	500	505	510

Lys Asn Leu Tyr His Ala Asp Gln Val Ala Pro Leu Leu Val Tyr Leu  
 515 520 525  
 Gly Thr Asp Asp Val Pro Val Thr Gly Glu Thr Ser Glu Ile Gly Gly  
 530 535 540  
 Gly Trp Ile Gly Asn Thr Arg Trp Gln Arg Ala Lys Gly Ala Val Ser  
 545 550 555 560  
 His Asp Glu His Thr Thr Val Glu Phe Ile Lys Glu His Leu Asn Glu  
 565 570 575  
 Ile Thr Asp Phe Thr Thr Asp Thr Glu Asn Pro Lys Ser Thr Thr Glu  
 580 585 590  
 Ser Ser Met Ala Ile Leu Ser Ala Val Gly Gly Asp Asp Asp Asp Asp  
 595 600 605  
 Asp Glu Asp Glu Glu Glu Asp Glu Gly Asp Glu Glu Glu Asp Glu Glu  
 610 615 620  
 Asp Glu Glu Glu Asp Asp Pro Val Trp Arg Phe Asp Asp Arg Asp Val  
 625 630 635 640  
 Ile Leu Tyr Asn Ile Ala Leu Gly Ala Thr Thr Lys Gln Leu Lys Tyr  
 645 650 655  
 Val Tyr Glu Asn Asp Ser Asp Phe Gln Val Ile Pro Thr Phe Gly His  
 660 665 670  
 Leu Ile Thr Phe Asn Ser Gly Lys Ser Gln Asn Ser Phe Ala Lys Leu  
 675 680 685  
 Leu Arg Asn Phe Asn Pro Met Leu Leu Leu His Gly Glu His Tyr Leu  
 690 695 700  
 Lys Val His Ser Trp Pro Pro Pro Thr Glu Gly Glu Ile Lys Thr Thr  
 705 710 715 720  
 Phe Glu Pro Ile Ala Thr Thr Pro Lys Gly Thr Asn Val Val Ile Val  
 725 730 735  
 His Gly Ser Lys Ser Val Asp Asn Lys Ser Gly Glu Leu Ile Tyr Ser  
 740 745 750  
 Asn Glu Ala Thr Tyr Phe Ile Arg Asn Cys Gln Ala Asp Asn Lys Val  
 755 760 765  
 Tyr Ala Asp Arg Pro Ala Phe Ala Thr Asn Gln Phe Leu Ala Pro Lys  
 770 775 780  
 Arg Ala Pro Asp Tyr Gln Val Asp Val Pro Val Ser Glu Asp Leu Ala  
 785 790 795 800  
 Ala Leu Tyr Arg Leu Ser Gly Asp Arg Asn Pro Leu His Ile Asp Pro  
 805 810 815

Asn Phe Ala Lys Gly Ala Lys Phe Pro Lys Pro Ile Leu His Gly Met  
                     820                    825                    830  
 5 Cys Thr Tyr Gly Leu Ser Ala Lys Ala Leu Ile Asp Lys Phe Gly Met  
                     835                    840                    845  
 Phe Asn Glu Ile Lys Ala Arg Phe Thr Gly Ile Val Phe Pro Gly Glu  
                     850                    855                    860  
 10 Thr Leu Arg Val Leu Ala Trp Lys Glu Ser Asp Asp Thr Ile Val Phe  
                     865                    870                    875                    880  
 Gln Thr His Val Val Asp Arg Gly Thr Ile Ala Ile Asn Asn Ala Ala  
 15                                      885                    890                    895  
 Ile Lys Leu Val Gly Asp Lys Ser Lys Leu  
                     900                    905

20 (2) INFORMATION FOR SEQ ID NO: 25:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 2737 base pairs  
 (B) TYPE: nucleic acid  
 25 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

30

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25:

GGATCCATGT CTCCAGTTGA TTTTAAAGAT AAAGTTGTGA TCATTACCGG TGCCGGTGGT 60  
 35 GGTTTGGGTA AATACTACTC CCTCGAATTT GCCAAGTTGG GCGCCAAAGT CGTCGTTAAC 120  
 GACTTGGGTG GTGCCTTGAA CGGTCAAGGT GGAAACTCCA AGGCCGCCGA CGTTGTCGTT 180  
 40 GACGAAATTG TCAAGAACGG TGGTGTGACC GTTGCCGATT ACAACAACGT CTTGGACGGT 240  
 GACAAGATTG TCGAAACCGC CGTCAAGAAC TTTGGTACTG TCCACGTTAT CATCAACAAT 300  
 GCCGGTATCT TGAGAGATGC CTCCATGAAG AAGATGACTG AAAAAGACTA CAAATTGGTC 360  
 45 ATTGACGTGC ACTTGAACGG TGCCTTTGCC GTCACCAAGG CTGCTTG GCC ATACTTCCAA 420  
 AAGCAAAAAT ACGGTAGAAT TGTCAACACA TCCTCCCCAG CTGGTTTGTA CGGTAACTTT 480  
 50 GGTCAAGCCA ACTACGCCTC CGCCAAGTCT GCTTTGTTGG GATTCGCTGA AACCTTGGCC 540  
 AAGGAAGGTG CCAAATACAA CATCAAGGCC AACGCCATTG CTCCGTTGGC CAGATCAAGA 600  
 ATGACTGAAT CTATCTTGCC ACCTCCAATG TTGGAAAAAT TGGGCCCTGA AAAGGTTGCC 660  
 55 CCATTGGTCT TGTATTTGTC GTCAGCTGAA AACGAATTGA CTGGTCAATT CTTTGAAGTT 720

	GCTGCTGGCT	TTTACGCTCA	GATCAGATGG	GAAAGATCCG	GTGGTGTCTT	GTTCAAGCCA	780
	GATCAATCCT	TCACCGCTGA	GGTTGTTGCT	AAGAGATTCT	CTGAAATCCT	TGATTATGAC	840
5	GACTCTAGGA	AGCCAGAATA	CTTGAAGAAC	CAATACCCAT	TCATGTTGAA	CGACTACGCC	900
	ACTTTGACCA	ACGAAGCTAG	AAAGTTGCCA	GCTAACGATG	CTTCTGGTGC	TCCAACGTGC	960
10	TCCTTGAAGG	ACAAGGTTGT	TTTGATCACC	GGTGCCGGTG	CTGGTTTGGG	TAAAGAATAC	1020
	GCCAAGTGGT	TCGCCAAGTA	CGGTGCCAAG	GTTGTTGTTA	ACGACTTCAA	GGATGCTACC	1080
	AAGACCGTTG	ACGAAATCAA	AGCCGCTGGT	GGTGAAGCTT	GGCCAGATCA	ACACGATGTT	1140
15	GCCAAGGACT	CCGAAGCTAT	CATCAAGAAT	GTCATTGACA	AGTACGGTAC	CATTGATATC	1200
	TTGGTCAACA	ACGCCGGTAT	CTTGAGAGAC	AGATCCTTTG	CCAAGATGTC	CAAGCAAGAA	1260
20	TGGGACTCTG	TCCAACAAGT	CCACTTGATT	GGTACTTTCA	ACTTGAGCAG	ATTGGCATGG	1320
	CCATACTTTG	TTGAAAAACA	ATTTGGTAGA	ATCATCAACA	TTACCTCCAC	CAGTGGTATC	1380
	TACGGTAACT	TTGGTCAAGC	CAACTACTCG	TCTTCTAAGG	CTGGTATCTT	GGGTTTGTCC	1440
25	AAGACCATGG	CCATTGAAGG	TGCTAAGAAT	AACATTAAGG	TCAACATTGT	TGCTCCACAC	1500
	GCTGAAACTG	CCATGACCTT	GACCATCTTC	AGAGAACAAG	ACAAGAACTT	GTACCACGCT	1560
30	GACCAAGTTG	CTCCATTGTT	GGTCTACTTG	GGTACTGACG	ATGTCCCAGT	CACCGGTGAA	1620
	ACTTCCGAAA	TCGGTGGTGG	TTGGATCGGT	AACACCAGAT	GGCAAAGAGC	CAAGGGTGCT	1680
	GTCTCCCACG	ACGAACACAC	CACTGTTGAA	TTCATCAAGG	AGCACTTGAA	CGAAATCACT	1740
35	GACTTCACCA	CTGACACTGA	AAATCCAAAA	TCTACCACCG	AATCCTCCAT	GGCTATCTTG	1800
	TCTGCCGTTG	GTGGTGATGA	CGATGATGAT	GACGAAGACG	AAGAAGAAGA	CGAAGGTGAT	1860
40	GAAGAAGAAG	ACGAAGAAGA	CGAAGAAGAA	GACGATCCAG	TCTGGAGATT	CGACGACAGA	1920
	GATGTTATCT	TGTACAACAT	TGCCCTTGGT	GCCACCACCA	AGCAATTGAA	GTACGTCTAC	1980
	GAAAACGACT	CTGACTTCCA	AGTCATTCCA	ACCTTTGGTC	ACTTGATCAC	CTTCAACTCT	2040
45	GGTAAGTCAC	AAAACCTCCT	TGCCAAGTTG	TTGCGTAACT	TCAACCCAAT	GTTGTTGTTG	2100
	CACGGTGAAC	ACTACTTGAA	GGTGCACAGC	TGGCCACCAC	CAACCGAAGG	TGAAATCAAG	2160
50	ACCACTTTTCG	AACCAATTGC	CACTACTCCA	AAGGGTACCA	ACGTTGTTAT	TGTTACGGT	2220
	TCCAAATCTG	TTGACAACAA	GTCTGGTGAA	TTGATTTACT	CCAACGAAGC	CACTTACTTC	2280
	ATCAGAAACT	GTCAAGCCGA	CAACAAGGTC	TACGCTGACC	GTCCAGCATT	CGCCACCAAC	2340
55	CAATTCTTGG	CACCAAAGAG	AGCCCCAGAC	TACCAAGTTG	ACGTTCCAGT	CAGTGAAGAC	2400

TTGGCTGCTT TGTACCGTTT GTCTGGTGAC AGAAACCCAT TGCACATTGA TCCAAACTTT 2460  
 GCTAAAGGTG CCAAGTTCCC TAAGCCAATC TTACACGGTA TGTGCACTTA TGGTTTGAGT 2520  
 5 GCTAAGGCTT TGATTGACAA GTTTGGTATG TTCAACGAAA TCAAGGCCAG ATTCACCGGT 2580  
 ATTGTCTTCC CAGGTGAAAC CTTGAGAGTC TTGGCATGGA AGGAAAGCGA TGACACTATT 2640  
 10 GTCTTCCAAA CTCATGTTGT TGATAGAGGT ACTATTGCCA TTAACAACGC TGCTATTAAG 2700  
 TTAGTCGGTG ACAAATGAAA GATCGAATGA AGGATCC 2737

## (2) INFORMATION FOR SEQ ID NO: 26:

15

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 903 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS:  
 20 (D) TOPOLOGY: linear

25

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26:

Met Ser Pro Val Asp Phe Lys Asp Lys Val Val Ile Ile Thr Gly Ala  
 1 5 10 15  
 30 Gly Gly Gly Leu Gly Lys Tyr Tyr Ser Leu Glu Phe Ala Lys Leu Gly  
 20 25 30  
 Ala Lys Val Val Val Asn Asp Leu Gly Gly Ala Leu Asn Gly Gln Gly  
 35 35 40 45  
 Gly Asn Ser Lys Ala Ala Asp Val Val Val Asp Glu Ile Val Lys Asn  
 50 55 60  
 40 Gly Gly Val Ala Val Ala Asp Tyr Asn Asn Val Leu Asp Gly Asp Lys  
 65 70 75 80  
 Ile Val Glu Thr Ala Val Lys Asn Phe Gly Thr Val His Val Ile Ile  
 85 90 95  
 45 Asn Asn Ala Gly Ile Leu Arg Asp Ala Ser Met Lys Lys Met Thr Glu  
 100 105 110  
 Lys Asp Tyr Lys Leu Val Ile Asp Val His Leu Asn Gly Ala Phe Ala  
 50 115 120 125  
 Val Thr Lys Ala Ala Trp Pro Tyr Phe Gln Lys Gln Lys Tyr Gly Arg  
 130 135 140  
 55 Ile Val Asn Thr Ser Ser Pro Ala Gly Leu Tyr Gly Asn Phe Gly Gln  
 145 150 155 160



	Ala	Asn	Tyr	Ala	Ser	Ala	Lys	Ser	Ala	Leu	Leu	Gly	Phe	Ala	Glu	Thr	
					165					170					175		
5	Leu	Ala	Lys	Glu	Gly	Ala	Lys	Tyr	Asn	Ile	Lys	Ala	Asn	Ala	Ile	Ala	
				180					185					190			
	Pro	Leu	Ala	Arg	Ser	Arg	Met	Thr	Glu	Ser	Ile	Leu	Pro	Pro	Pro	Met	
			195					200					205				
10	Leu	Glu	Lys	Leu	Gly	Pro	Glu	Lys	Val	Ala	Pro	Leu	Val	Leu	Tyr	Leu	
		210					215					220					
	Ser	Ser	Ala	Glu	Asn	Glu	Leu	Thr	Gly	Gln	Phe	Phe	Glu	Val	Ala	Ala	
15	225					230					235					240	
	Gly	Phe	Tyr	Ala	Gln	Ile	Arg	Trp	Glu	Arg	Ser	Gly	Gly	Val	Leu	Phe	
				245						250					255		
20	Lys	Pro	Asp	Gln	Ser	Phe	Thr	Ala	Glu	Val	Val	Ala	Lys	Arg	Phe	Ser	
				260					265					270			
	Glu	Ile	Leu	Asp	Tyr	Asp	Asp	Ser	Arg	Lys	Pro	Glu	Tyr	Leu	Lys	Asn	
			275					280					285				
25	Gln	Tyr	Pro	Phe	Met	Leu	Asn	Asp	Tyr	Ala	Thr	Leu	Thr	Asn	Glu	Ala	
		290					295					300					
	Arg	Lys	Leu	Pro	Ala	Asn	Asp	Ala	Ser	Gly	Ala	Pro	Thr	Val	Ser	Leu	
30	305					310					315					320	
	Lys	Asp	Lys	Val	Val	Leu	Ile	Thr	Gly	Ala	Gly	Ala	Gly	Leu	Gly	Lys	
				325						330				335			
35	Glu	Tyr	Ala	Lys	Trp	Phe	Ala	Lys	Tyr	Gly	Ala	Lys	Val	Val	Val	Asn	
				340					345					350			
	Asp	Phe	Lys	Asp	Ala	Thr	Lys	Thr	Val	Asp	Glu	Ile	Lys	Ala	Ala	Gly	
			355					360					365				
40	Gly	Glu	Ala	Trp	Pro	Asp	Gln	His	Asp	Val	Ala	Lys	Asp	Ser	Glu	Ala	
		370					375					380					
	Ile	Ile	Lys	Asn	Val	Ile	Asp	Lys	Tyr	Gly	Thr	Ile	Asp	Ile	Leu	Val	
45	385					390					395					400	
	Asn	Asn	Ala	Gly	Ile	Leu	Arg	Asp	Arg	Ser	Phe	Ala	Lys	Met	Ser	Lys	
				405						410					415		
50	Gln	Glu	Trp	Asp	Ser	Val	Gln	Gln	Val	His	Leu	Ile	Gly	Thr	Phe	Asn	
				420					425					430			
	Leu	Ser	Arg	Leu	Ala	Trp	Pro	Tyr	Phe	Val	Glu	Lys	Gln	Phe	Gly	Arg	
			435					440					445				
55	Ile	Ile	Asn	Ile	Thr	Ser	Thr	Ser	Gly	Ile	Tyr	Gly	Asn	Phe	Gly	Gln	

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	Asn	Glu	Ala	Thr	Tyr	Phe	Ile	Arg	Asn	Cys	Gln	Ala	Asp	Asn	Lys	Val	
			755					760					765				
5	Tyr	Ala	Asp	Arg	Pro	Ala	Phe	Ala	Thr	Asn	Gln	Phe	Leu	Ala	Pro	Lys	
		770					775					780					
	Arg	Ala	Pro	Asp	Tyr	Gln	Val	Asp	Val	Pro	Val	Ser	Glu	Asp	Leu	Ala	
	785					790					795					800	
10	Ala	Leu	Tyr	Arg	Leu	Ser	Gly	Asp	Arg	Asn	Pro	Leu	His	Ile	Asp	Pro	
					805					810					815		
	Asn	Phe	Ala	Lys	Gly	Ala	Lys	Phe	Pro	Lys	Pro	Ile	Leu	His	Gly	Met	
				820					825					830			
15	Cys	Thr	Tyr	Gly	Leu	Ser	Ala	Lys	Ala	Leu	Ile	Asp	Lys	Phe	Gly	Met	
			835					840					845				
	Phe	Asn	Glu	Ile	Lys	Ala	Arg	Phe	Thr	Gly	Ile	Val	Phe	Pro	Gly	Glu	
20		850					855					860					
	Thr	Leu	Arg	Val	Leu	Ala	Trp	Lys	Glu	Ser	Asp	Asp	Thr	Ile	Val	Phe	
	865					870					875					880	
25	Gln	Thr	His	Val	Val	Asp	Arg	Gly	Thr	Ile	Ala	Ile	Asn	Asn	Ala	Ala	
				885						890						895	
	Ile	Lys	Leu	Val	Gly	Asp	Lys										
				900													

**WHAT IS CLAIMED IS:**

1. A non-naturally occurring fusion protein comprising:  
a peroxisome targeting protein subunit; and  
a polyhydroxyalkanoate synthase protein subunit.
- 5 2. The fusion protein of claim 1, wherein the peroxisome targeting subunit is PTS2.
3. The fusion protein of claim 1, wherein the peroxisome targeting subunit comprises a tripeptide, wherein:  
10 the first amino acid in the N-terminus to C-terminus direction is S, A, or P;  
the second amino acid in the N-terminus to C-terminus direction is K, R, S, or H;  
and  
the third amino acid in the N-terminus to C-terminus direction is L, M, I, or F.
- 15 4. The fusion protein of claim 3, wherein the peroxisome targeting subunit comprises ARM, SRM, SKL, ARL, SRL, PSI, or PRM.
5. The fusion protein of claim 1, wherein the peroxisome targeting subunit is at least 70% identical to SEQ ID NO:14.
- 20 6. The fusion protein of claim 5, wherein the peroxisome targeting protein subunit is at least 80% identical to SEQ ID NO:14.
7. The fusion protein of claim 6, wherein the peroxisome targeting protein subunit is at  
25 least 90% identical to SEQ ID NO:14.
8. The fusion protein of claim 7, wherein the peroxisome targeting protein subunit is SEQ ID NO:14.
- 30 9. The fusion protein of claim 1, wherein the polyhydroxyalkanoate synthase protein subunit is a *Pseudomonas* subunit.

10. The fusion protein of claim 9, wherein the *Pseudomonas* subunit is a *Pseudomonas aeruginosa* subunit.
11. The fusion protein of claim 10, wherein the polyhydroxyalkanoate synthase protein subunit is a PHAC1 subunit.
- 5 12. The fusion protein of claim 11, wherein the polyhydroxyalkanoate synthase protein subunit is at least 70% identical to SEQ ID NO:2.
- 10 13. The fusion protein of claim 12, wherein the polyhydroxyalkanoate synthase protein subunit is at least 80% identical to SEQ ID NO:2.
14. The fusion protein of claim 13, wherein the polyhydroxyalkanoate synthase protein subunit is at least 90% identical to SEQ ID NO:2.
- 15 15. The fusion protein of claim 14, wherein the polyhydroxyalkanoate synthase protein subunit is SEQ ID NO:2.
16. The fusion protein of claim 10, wherein the polyhydroxyalkanoate synthase protein subunit is a PHAC2 subunit.
- 20 17. The fusion protein of claim 16, wherein the polyhydroxyalkanoate synthase protein subunit is at least 70% identical to SEQ ID NO:4.
18. The fusion protein of claim 17, wherein the polyhydroxyalkanoate synthase protein subunit is at least 80% identical to SEQ ID NO:4.
- 25 19. The fusion protein of claim 18, wherein the polyhydroxyalkanoate synthase protein subunit is at least 90% identical to SEQ ID NO:4.
- 30 20. The fusion protein of claim 19, wherein the polyhydroxyalkanoate synthase protein subunit is SEQ ID NO:4.

21. The fusion protein of claim 1, wherein the polyhydroxyalkanoate synthase protein subunit is at least 70% identical to SEQ ID NO:18 or SEQ ID NO:20.
22. The fusion protein of claim 21, wherein the polyhydroxyalkanoate synthase protein subunit is at least 80% identical to SEQ ID NO:18 or SEQ ID NO:20.
- 5 23. The fusion protein of claim 22, wherein the polyhydroxyalkanoate synthase protein subunit is at least 90% identical to SEQ ID NO:18 or SEQ ID NO:20.
24. The fusion protein of claim 23, comprising SEQ ID NO:18 or SEQ ID NO:20.
- 10 25. A nucleic acid segment encoding a non-naturally occurring fusion protein, the nucleic acid segment comprising:  
a nucleic acid sequence encoding a peroxisome targeting protein subunit; and  
a nucleic acid sequence encoding a polyhydroxyalkanoate synthase protein subunit.
- 15 26. The nucleic acid segment of claim 25, wherein the nucleic acid sequence encoding a peroxisome targeting protein subunit comprises at least a 6 contiguous nucleic acid sequence from SEQ ID NO:13.
- 20 27. The nucleic acid segment of claim 25, wherein the nucleic acid sequence encoding a peroxisome targeting protein subunit is at least 70% identical to SEQ ID NO:13.
28. The nucleic acid segment of claim 27, wherein the nucleic acid sequence encoding a peroxisome targeting protein subunit is at least 80% identical to SEQ ID NO:13.
- 25 29. The nucleic acid segment of claim 28, wherein the nucleic acid sequence encoding a peroxisome targeting protein subunit is at least 90% identical to SEQ ID NO:13.
30. The nucleic acid segment of claim 29, wherein the nucleic acid sequence encoding a peroxisome targeting protein subunit is SEQ ID NO:13.
- 30

31. The nucleic acid segment of claim 25, wherein the nucleic acid sequence encoding a peroxisome targeting protein subunit hybridizes to SEQ ID NO:13.
32. The nucleic acid segment of claim 25, wherein the nucleic acid sequence encoding a polyhydroxyalkanoate synthase protein subunit comprises at least a 6 contiguous nucleic acid sequence from:  
SEQ ID NO:1;  
SEQ ID NO:3;  
SEQ ID NO:15; or  
SEQ ID NO:16.
33. The nucleic acid segment of claim 25, wherein the nucleic acid sequence encoding a polyhydroxyalkanoate synthase protein subunit is at least 70% identical to:  
SEQ ID NO:1;  
SEQ ID NO:3;  
SEQ ID NO:15; or  
SEQ ID NO:16.
34. The nucleic acid segment of claim 33, wherein the nucleic acid sequence encoding a polyhydroxyalkanoate synthase protein subunit is at least 80% identical to:  
SEQ ID NO:1;  
SEQ ID NO:3;  
SEQ ID NO:15; or  
SEQ ID NO:16.
35. The nucleic acid segment of claim 34, wherein the nucleic acid sequence encoding a polyhydroxyalkanoate synthase protein subunit is at least 90% identical to:  
SEQ ID NO:1;  
SEQ ID NO:3;  
SEQ ID NO:15; or  
SEQ ID NO:16.

36. The nucleic acid segment of claim 35, wherein the nucleic acid sequence encoding a polyhydroxyalkanoate synthase protein subunit is:  
SEQ ID NO:1;  
SEQ ID NO:3;  
5 SEQ ID NO:15; or  
SEQ ID NO:16.
37. The nucleic acid segment of claim 36, wherein the nucleic acid sequence encoding a polyhydroxyalkanoate synthase protein subunit is:  
SEQ ID NO:15; or  
10 SEQ ID NO:16.
38. The nucleic acid segment of claim 25, wherein the nucleic acid sequence encoding a polyhydroxyalkanoate synthase protein subunit hybridizes to:  
SEQ ID NO:1;  
15 SEQ ID NO:3;  
SEQ ID NO:15; or  
SEQ ID NO:16.
39. The nucleic acid segment of claim 25, wherein the peroxisome targeting protein subunit is PTS2.  
20
40. The nucleic acid segment of claim 25, wherein the peroxisome targeting protein subunit comprises a tripeptide, the tripeptide having:  
a first amino acid in the N-terminus to C-terminus direction being S, A, or P;  
25 a second amino acid in the N-terminus to C-terminus direction being K, R, S, or H;  
and  
a third amino acid in the N-terminus to C-terminus direction being L, M, I, or F.
41. The nucleic acid segment of claim 40, wherein the peroxisome targeting subunit comprises ARM, SRM, SKL, ARL, SRL, PSI, or PRM.  
30



42. The nucleic acid segment of claim 25, wherein the nucleic acid sequence encoding a polyhydroxyalkanoate synthase protein subunit encodes at least a 5 contiguous amino acid sequence from:  
SEQ ID NO:2; or  
5 SEQ ID NO:4.
43. The nucleic acid segment of claim 25, wherein the nucleic acid sequence encoding a polyhydroxyalkanoate synthase protein subunit encodes an amino acid sequence at least about 70% identical to:  
10 SEQ ID NO:2; or  
SEQ ID NO:4.
44. The nucleic acid segment of claim 43, wherein the nucleic acid sequence encoding a polyhydroxyalkanoate synthase protein subunit encodes an amino acid sequence at least about 80% identical to:  
15 SEQ ID NO:2; or  
SEQ ID NO:4.
45. The nucleic acid segment of claim 44, wherein the nucleic acid sequence encoding a polyhydroxyalkanoate synthase protein subunit encodes an amino acid sequence at least about 90% identical to:  
20 SEQ ID NO:2; or  
SEQ ID NO:4.
46. The nucleic acid segment of claim 45, wherein the nucleic acid sequence encoding a polyhydroxyalkanoate synthase protein subunit encodes:  
25 SEQ ID NO:2; or  
SEQ ID NO:4.
47. A recombinant vector comprising in the 5' to 3' direction:  
30

- a) a promoter that directs transcription of a structural nucleic acid sequence encoding a non-naturally occurring fusion protein, wherein the fusion protein comprises:
- i) a peroxisome targeting protein subunit; and
  - ii) a polyhydroxyalkanoate synthase protein subunit.
- b) a structural nucleic acid sequence encoding a non-naturally occurring fusion protein, wherein the fusion protein comprises:
- i) a peroxisome targeting protein subunit; and
  - ii) a polyhydroxyalkanoate synthase protein subunit; and
- c) a 3' transcription terminator.
48. The recombinant vector of claim 47, further comprising a 3' polyadenylation signal sequence that directs the addition of polyadenylate nucleotides to the 3' end of RNA transcribed from the structural nucleic acid coding sequence.
49. The recombinant vector of claim 47, further comprising a selectable marker.
50. The recombinant vector of claim 49, wherein the selectable marker is a kanamycin resistance marker, a hygromycin resistance marker, or a herbicide resistance marker.
51. The recombinant vector of claim 47, wherein the promoter is constitutive.
52. The recombinant vector of claim 51, wherein the promoter is CaMV35S, enhanced CaMV35S, FMV, mas, nos, or ocs.
53. The recombinant vector of claim 47, wherein the promoter is inducible.
54. The recombinant vector of claim 53, wherein the promoter is tac, salicylic acid induced, polyacrylic acid induced, safener induced, heat shock promoter, nitrate induced, hormone induced, or light induced.
55. The recombinant vector of claim 47, wherein the promoter is tissue specific.

56. The recombinant vector of claim 55, wherein the promoter is the  $\beta$ -conglycinin 7S promoter, napin promoter, phaseolin promoter, zein promoter, soybean trypsin inhibitor promoter, ACP promoter, stearyl-ACP desaturase promoter, or oleosin promoter.
- 5 57. The recombinant vector of claim 47, wherein the nucleic acid sequence encoding a peroxisome targeting protein subunit comprises at least a 6 contiguous nucleic acid sequence from SEQ ID NO:13.
58. The recombinant vector of claim 47, wherein the nucleic acid sequence encoding a peroxisome targeting protein subunit is at least 70% identical to SEQ ID NO:13.
- 10 59. The recombinant vector of claim 58, wherein the nucleic acid sequence encoding a peroxisome targeting protein subunit is at least 80% identical to SEQ ID NO:13.
60. The recombinant vector of claim 59, wherein the nucleic acid sequence encoding a peroxisome targeting protein subunit is at least 90% identical to SEQ ID NO:13.
61. The recombinant vector of claim 60, wherein the nucleic acid sequence encoding a peroxisome targeting protein subunit is SEQ ID NO:13.
- 15 62. The recombinant vector of claim 47, wherein the nucleic acid sequence encoding a peroxisome targeting protein subunit hybridizes to SEQ ID NO:13.
63. The recombinant vector of claim 47, wherein the peroxisome targeting protein subunit is PTS2.
- 20 64. The recombinant vector of claim 47, wherein the peroxisome targeting protein subunit comprises a tripeptide, the tripeptide having:  
a first amino acid in the N-terminus to C-terminus direction being S, A, or P;  
a second amino acid in the N-terminus to C-terminus direction being K, R, S, or H;  
and  
25 a third amino acid in the N-terminus to C-terminus direction being L, M, I, or F.

65. The recombinant vector of claim 64, wherein the peroxisome targeting subunit comprises ARM, SRM, SKL, ARL, SRL, PSI, or PRM.
66. The recombinant vector of claim 47, wherein the polyhydroxyalkanoate synthase protein subunit is a *Pseudomonas* subunit.
67. The recombinant vector of claim 66, wherein the *Pseudomonas* subunit is a *Pseudomonas aeruginosa* subunit.
68. The recombinant vector of claim 47, wherein the nucleic acid sequence encoding a polyhydroxyalkanoate synthase protein subunit comprises at least a 6 contiguous nucleic acid sequence from:  
SEQ ID NO:1;  
SEQ ID NO:3;  
SEQ ID NO:15; or  
SEQ ID NO:16.
69. The recombinant vector of claim 47, wherein the nucleic acid sequence encoding a polyhydroxyalkanoate synthase protein subunit is at least 70% identical to:  
SEQ ID NO:1;  
SEQ ID NO:3;  
SEQ ID NO:15; or  
SEQ ID NO:16.
70. The recombinant vector of claim 69, wherein the nucleic acid sequence encoding a polyhydroxyalkanoate synthase protein subunit is at least 80% identical to:  
SEQ ID NO:1;  
SEQ ID NO:3;  
SEQ ID NO:15; or  
SEQ ID NO:16.

71. The recombinant vector of claim 70, wherein the nucleic acid sequence encoding a polyhydroxyalkanoate synthase protein subunit is at least 90% identical to:  
SEQ ID NO:1;  
SEQ ID NO:3;  
5 SEQ ID NO:15; or  
SEQ ID NO:16.
72. The recombinant vector of claim 71, wherein the nucleic acid sequence encoding a polyhydroxyalkanoate synthase protein subunit is:  
10 SEQ ID NO:1;  
SEQ ID NO:3;  
SEQ ID NO:15; or  
SEQ ID NO:16.
- 15 73. The recombinant vector of claim 72, wherein the nucleic acid sequence encoding a polyhydroxyalkanoate synthase protein subunit is:  
SEQ ID NO:15; or  
SEQ ID NO:16.
- 20 74. The recombinant vector of claim 47, wherein the nucleic acid sequence encoding a polyhydroxyalkanoate synthase protein subunit hybridizes to:  
SEQ ID NO:1;  
SEQ ID NO:3;  
SEQ ID NO:15; or  
25 SEQ ID NO:16.
75. The recombinant vector of claim 47, wherein the nucleic acid sequence encoding a polyhydroxyalkanoate synthase protein subunit encodes at least a 5 contiguous amino acid sequence from:  
30 SEQ ID NO:2; or  
SEQ ID NO:4.

76. The recombinant vector of claim 47, wherein the nucleic acid sequence encoding a polyhydroxyalkanoate synthase protein subunit encodes an amino acid sequence at least about 70% identical to:  
SEQ ID NO:2; or  
5 SEQ ID NO:4.
77. The recombinant vector of claim 76, wherein the nucleic acid sequence encoding a polyhydroxyalkanoate synthase protein subunit encodes an amino acid sequence at least about 80% identical to:  
10 SEQ ID NO:2; or  
SEQ ID NO:4.
78. The recombinant vector of claim 77, wherein the nucleic acid sequence encoding a polyhydroxyalkanoate synthase protein subunit encodes an amino acid sequence at least about 90% identical to:  
15 SEQ ID NO:2; or  
SEQ ID NO:4.
79. The recombinant vector of claim 78, wherein the nucleic acid sequence encoding a polyhydroxyalkanoate synthase protein subunit encodes:  
20 SEQ ID NO:2; or  
SEQ ID NO:4.
80. The recombinant vector of claim 47, wherein the structural nucleic acid sequence comprises:  
SEQ ID NO:17; or  
SEQ ID NO:19.
- 25 81. The recombinant vector of claim 47, wherein the structural nucleic acid sequence encodes:  
SEQ ID NO:18; or  
SEQ ID NO:20.

82. A recombinant host cell comprising a nucleic acid segment encoding a non-naturally occurring fusion protein, wherein the nucleic acid segment comprises:  
a nucleic acid sequence encoding a peroxisome targeting protein subunit; and  
a nucleic acid sequence encoding a polyhydroxyalkanoate synthase protein subunit.
- 5
83. The recombinant host cell of claim 82, wherein the recombinant host cell is a fungal cell.
84. The recombinant host cell of claim 83, wherein the fungal cell is a  
10 *Schizosaccharomyces pombe*, *Streptomyces rimofaciens*, *Fusarium*, *Aspergillus niger*, or *Saccharomyces cerevisiae* cell.
85. The recombinant host cell of claim 82, wherein the recombinant host cell is a plant cell.
- 15
86. The recombinant host cell of claim 85, wherein the plant cell is an alfalfa, banana, barley, bean, cabbage, canola/oilseed rape, carrot, castorbean, celery, clover, coconut, corn, cotton, cucumber, linseed, melon, olive, palm, parsnip, pea, peanut, pepper, potato, potato, radish, rapeseed, rice, soybean, spinach, sunflower, tobacco,  
20 tomato, or wheat cell.
87. The recombinant host cell of claim 82, further comprising a nucleic acid segment encoding an acyl-ACP thioesterase.
- 25
88. The recombinant host cell of claim 82, further comprising a nucleic acid segment encoding a fatty acyl hydroxylase.
89. The recombinant host cell of claim 82, further comprising a nucleic acid segment encoding a yeast multifunctional protein (MFP).
- 30

90. The recombinant host cell of claim 82, further comprising a nucleic acid segment encoding a hydroxyacyl-CoA epimerase.

91. A genetically transformed plant cell comprising in the 5' to 3' direction:

- a) a promoter to direct transcription of a structural nucleic acid sequence encoding a non-naturally occurring fusion protein, wherein the structural nucleic acid sequence comprises:
  - i) a nucleic acid sequence encoding a peroxisome targeting protein subunit; and
  - ii) a nucleic acid sequence encoding a polyhydroxyalkanoate synthase protein subunit;
- b) a structural nucleic acid sequence encoding a non-naturally occurring fusion protein, wherein the structural nucleic acid sequence comprises:
  - i) a nucleic acid sequence encoding a peroxisome targeting protein subunit; and
  - ii) a nucleic acid sequence encoding a polyhydroxyalkanoate synthase protein subunit;
- c) a 3' transcription terminator sequence; and
- d) a 3' polyadenylation signal sequence that directs the addition of polyadenylate nucleotides to the 3' end of RNA transcribed from the structural nucleic acid coding sequence.

92. The genetically transformed plant cell of claim 91, wherein the plant cell is an alfalfa, banana, barley, bean, cabbage, canola/oilseed rape, carrot, castorbean, celery, clover, coconut, corn, cotton, cucumber, linseed, melon, olive, palm, parsnip, pea, peanut, pepper, potato, radish, rapeseed, rice, soybean, spinach, sunflower, tobacco, tomato, or wheat cell.

93. The genetically transformed plant cell of claim 91, further comprising a nucleic acid segment encoding an acyl-ACP thioesterase.



94. The genetically transformed plant cell of claim 91, further comprising a nucleic acid segment encoding a fatty acyl hydroxylase.
95. The genetically transformed plant cell of claim 91, further comprising a nucleic acid segment encoding a yeast multifunctional protein (MFP).
96. The genetically transformed plant cell of claim 91, further comprising a nucleic acid segment encoding a hydroxyacyl-CoA epimerase.
97. A genetically transformed plant comprising in the 5' to 3' direction:
- a) a promoter to direct transcription of a structural nucleic acid sequence encoding a non-naturally occurring fusion protein, wherein the structural nucleic acid sequence comprises:
    - i) a nucleic acid sequence encoding a peroxisome targeting protein subunit; and
    - ii) a nucleic acid sequence encoding a polyhydroxyalkanoate synthase protein subunit;
  - b) a structural nucleic acid sequence encoding a non-naturally occurring fusion protein, wherein the structural nucleic acid sequence comprises:
    - i) a nucleic acid sequence encoding a peroxisome targeting protein subunit; and
    - ii) a nucleic acid sequence encoding a polyhydroxyalkanoate synthase protein subunit;
  - c) a 3' transcription terminator sequence; and
  - d) a 3' polyadenylation signal sequence that directs the addition of polyadenylate nucleotides to the 3' end of RNA transcribed from the structural nucleic acid coding sequence.
98. The genetically transformed plant of claim 97, wherein the plant is an alfalfa, banana, barley, bean, cabbage, canola/oilseed rape, carrot, castorbean, celery, clover, coconut, corn, cotton, cucumber, linseed, melon, olive, palm, parsnip, pea, peanut,

pepper, potato, potato, radish, rapeseed, rice, soybean, spinach, sunflower, tobacco, tomato, or wheat plant.

- 5
99. The genetically transformed plant of claim 97, wherein the promoter is constitutive.
100. The genetically transformed plant of claim 99, wherein the promoter is CaMV35S, enhanced CaMV35S, FMV, mas, nos, or ocs.
101. The genetically transformed plant of claim 97, wherein the promoter is inducible.
- 10
102. The genetically transformed plant of claim 101, wherein the promoter is tac, salicylic acid induced, polyacrylic acid induced, safener induced, heat shock promoter, nitrate induced, hormone induced, or light induced.
103. The genetically transformed plant of claim 97, wherein the promoter is tissue specific.
- 15
104. The genetically transformed plant of claim 103, wherein the promoter is the  $\beta$ -conglycinin 7S promoter, napin promoter, phaseolin promoter, zein promoter, soybean trypsin inhibitor promoter, ACP promoter, stearyl-ACP desaturase promoter, or oleosin promoter.
- 20
105. The genetically transformed plant of claim 97, further comprising a nucleic acid segment encoding an acyl-ACP thioesterase.
- 25
106. The genetically transformed plant of claim 97, further comprising a nucleic acid segment encoding a fatty acyl hydroxylase.
107. The genetically transformed plant of claim 97, further comprising a nucleic acid segment encoding a yeast multifunctional protein (MFP).
- 30

108. The genetically transformed plant of claim 97, further comprising a nucleic acid segment encoding a hydroxyacyl-CoA epimerase.
109. A method of preparing host cells useful to produce a non-naturally occurring fusion protein comprising the steps of:
- a) selecting a host cell
  - b) transforming the selected host cell with a recombinant vector having a structural nucleic acid sequence encoding a non-naturally occurring fusion protein, wherein the structural nucleic acid sequence comprises:
    - i) a nucleic acid sequence encoding a peroxisome targeting protein subunit; and
    - ii) a nucleic acid sequence encoding a polyhydroxyalkanoate synthase protein subunit; and
  - c) obtaining transformed host cells.
110. The method of claim 109, wherein the vector further comprises a selectable marker.
111. The method of claim 110, wherein the selectable marker is a kanamycin resistance marker, a hygromycin resistance marker, or a herbicide resistance marker.
112. The method of claim 109, wherein the host cell is a fungal cell.
113. The method of claim 112, wherein the fungal cell is a *Schizosaccharomyces pombe*, *Streptomyces rimofaciens*, *Fusarium*, *Aspergillus niger*, or *Saccharomyces cerevisiae* cell.
114. The method of claim 109, wherein the host cell is a plant cell.
115. The method of claim 114, wherein the plant cell is an alfalfa, banana, barley, bean, cabbage, canola/oilseed rape, carrot, castorbean, celery, clover, coconut, corn, cotton, cucumber, linseed, melon, olive, palm, parsnip, pea, peanut, pepper, potato,

potato, radish, rapeseed, rice, soybean, spinach, sunflower, tobacco, tomato, or wheat cell.

- 5 116. A method of preparing a transformed plant useful to produce a non-naturally occurring fusion protein comprising the steps of:
- a) selecting a host plant cell
  - b) transforming the selected host plant cell with a recombinant vector having a structural nucleic acid sequence encoding a non-naturally occurring fusion protein, wherein the structural nucleic acid sequence comprises:
    - 10 i) a nucleic acid sequence encoding a peroxisome targeting protein subunit; and
    - ii) a nucleic acid sequence encoding a polyhydroxyalkanoate synthase protein subunit;
  - c) obtaining transformed host plant cells; and
  - 15 d) regenerating the transformed host plant cells.
117. The method of claim 116, wherein the vector further comprises a selectable marker.
118. The method of claim 117, wherein the selectable marker is a kanamycin resistance  
20 marker, a hygromycin resistance marker, or a herbicide resistance marker.
119. The method of claim 116, wherein the host plant cell is an an alfalfa, banana, barley, bean, cabbage, canola/oilseed rape, carrot, castorbean, celery, clover, coconut, corn, cotton, cucumber, linseed, melon, olive, palm, parsnip, pea, peanut, pepper, potato,  
25 potato, radish, rapeseed, rice, soybean, spinach, sunflower, tobacco, tomato, or wheat cell.
120. The plant produced by the method of claim 116.

121. A method for the preparation of a polyhydroxyalkanoate, comprising the steps of:
- a) obtaining a cell capable of producing a non-naturally occurring fusion protein, wherein the fusion protein comprises:
    - i) a peroxisome targeting protein subunit; and
    - ii) a polyhydroxyalkanoate synthase protein subunit;
  - b) establishing a culture of the cell; and
  - c) culturing the cell under conditions suitable for the production of the polyhydroxyalkanoate.
122. The method of claim 121, wherein the culture contains natural fatty acids, non-natural fatty acids, or mixtures thereof.
123. The method of claim 121, wherein the cell is a fungal cell.
124. The method of claim 123, wherein the fungal cell is a *Schizosaccharomyces pombe*, *Streptomyces rimofaciens*, *Fusarium*, *Aspergillus niger*, or *Saccharomyces cerevisiae* cell.
125. The method of claim 121, wherein the cell is a plant cell.
126. The method of claim 125, wherein the cell is an alfalfa, banana, barley, bean, cabbage, canola/oilseed rape, carrot, castorbean, celery, clover, coconut, corn, cotton, cucumber, linseed, melon, olive, palm, parsnip, pea, peanut, pepper, potato, radish, rapeseed, rice, soybean, spinach, sunflower, tobacco, tomato, or wheat cell.
127. The method of claim 121, wherein the polyhydroxyalkanoate comprises 3-hydroxyhexanoic acid (H:6), 3-hydroxyoctanoic acid (H:8), 3-hydroxydecanoic acid (H:10), 3-hydroxydodecanoic acid (H:12), 3-hydroxytetradecanoic acid (H:14), 3-hydroxyhexadecanoic acid (H:16), 3-hydroxyheptanoic acid (H:7), 3-hydroxynonanoic acid (H:9), 3-hydroxyundecanoic acid (H:11), 3-

hydroxytridecanoic acid (H:13), 3-hydroxyhexadecatrienoic acid (H16:3), 3-hydroxyhexadecadienoic acid (H16:2), 3-hydroxyhexadecenoic acid (H16:1), 3-hydroxytetradecatrienoic acid (H14:3), 3-hydroxytetradecadienoic acid (H14:2), 3-hydroxytetradecenoic acid (H14:1), 3-hydroxydodecadienoic acid (H12:2), 3-hydroxydodecenoic acid (H12:1), 3-hydroxyoctenoic acid (H8:1), 4-hydroxydecanoic acid, 8-methyl-3-hydroxynonanoic acid, or 6-methyl-3-hydroxyheptanoic acid monomers.

128. A method for the preparation of a polyhydroxyalkanoate, comprising the steps of:
- a) obtaining a plant capable of producing a non-naturally occurring fusion protein, wherein the fusion protein comprises:
    - i) a peroxisome targeting protein subunit; and
    - ii) a polyhydroxyalkanoate synthase protein subunit; and
  - b) growing the plant under conditions suitable for the production of the polyhydroxyalkanoate.
129. The method of claim 128, further comprising supplementing the plant with natural fatty acids, non-natural fatty acids, or mixtures thereof.
130. The method of claim 128, wherein the plant is an alfalfa, banana, barley, bean, cabbage, canola/oilseed rape, carrot, castorbean, celery, clover, coconut, corn, cotton, cucumber, linseed, melon, olive, palm, parsnip, pea, peanut, pepper, potato, potato, radish, rapeseed, rice, soybean, spinach, sunflower, tobacco, tomato, or wheat plant.
131. The method of claim 128, wherein the polyhydroxyalkanoate comprises 3-hydroxyhexanoic acid (H:6), 3-hydroxyoctanoic acid (H:8), 3-hydroxydecanoic acid (H:10), 3-hydroxydodecanoic acid (H:12), 3-hydroxytetradecanoic acid (H:14), 3-hydroxyhexadecanoic acid (H:16), 3-hydroxyheptanoic acid (H:7), 3-hydroxynonanoic acid (H9), 3-hydroxyundecanoic acid (H:11), 3-hydroxytridecanoic acid (H:13), 3-hydroxyhexadecatrienoic acid (H16:3), 3-

hydroxyhexadecadienoic acid (H16:2), 3-hydroxyhexadecenoic acid (H16:1), 3-hydroxytetradecatrienoic acid (H14:3), 3-hydroxytetradecadienoic acid (H14:2), 3-hydroxytetradecenoic acid (H14:1), 3-hydroxydodecadienoic acid (H12:2), 3-hydroxydodecenoic acid (H12:1), 3-hydroxyoctenoic acid (H8:1), 4-hydroxydecanoic acid, 8-methyl-3-hydroxynonanoic acid, or 6-methyl-3-hydroxyheptanoic acid monomers.

132. A plant containing a polyhydroxyalkanoate, wherein the polyhydroxyalkanoate comprises 3-hydroxyhexanoic acid (H:6), 3-hydroxyoctanoic acid (H:8), 3-hydroxydecanoic acid (H:10), 3-hydroxydodecanoic acid (H:12), 3-hydroxytetradecanoic acid (H:14), 3-hydroxyhexadecanoic acid (H:16), 3-hydroxyheptanoic acid (H:7), 3-hydroxynonanoic acid (H9), 3-hydroxyundecanoic acid (H:11), 3-hydroxytridecanoic acid (H:13), 3-hydroxyhexadecatrienoic acid (H16:3), 3-hydroxyhexadecadienoic acid (H16:2), 3-hydroxyhexadecenoic acid (H16:1), 3-hydroxytetradecatrienoic acid (H14:3), 3-hydroxytetradecadienoic acid (H14:2), 3-hydroxytetradecenoic acid (H14:1), 3-hydroxydodecadienoic acid (H12:2), 3-hydroxydodecenoic acid (H12:1), 3-hydroxyoctenoic acid (H8:1), 4-hydroxydecanoic acid, 8-methyl-3-hydroxynonanoic acid, or 6-methyl-3-hydroxyheptanoic acid monomers.

20

133. A polyhydroxyalkanoate comprising 3-hydroxyhexadecatrienoic acid (H16:3), 3-hydroxyhexadecadienoic acid (H16:2), 3-hydroxytetradecatrienoic acid (H14:3), or 3-hydroxydodecadienoic acid (H12:2) monomers.

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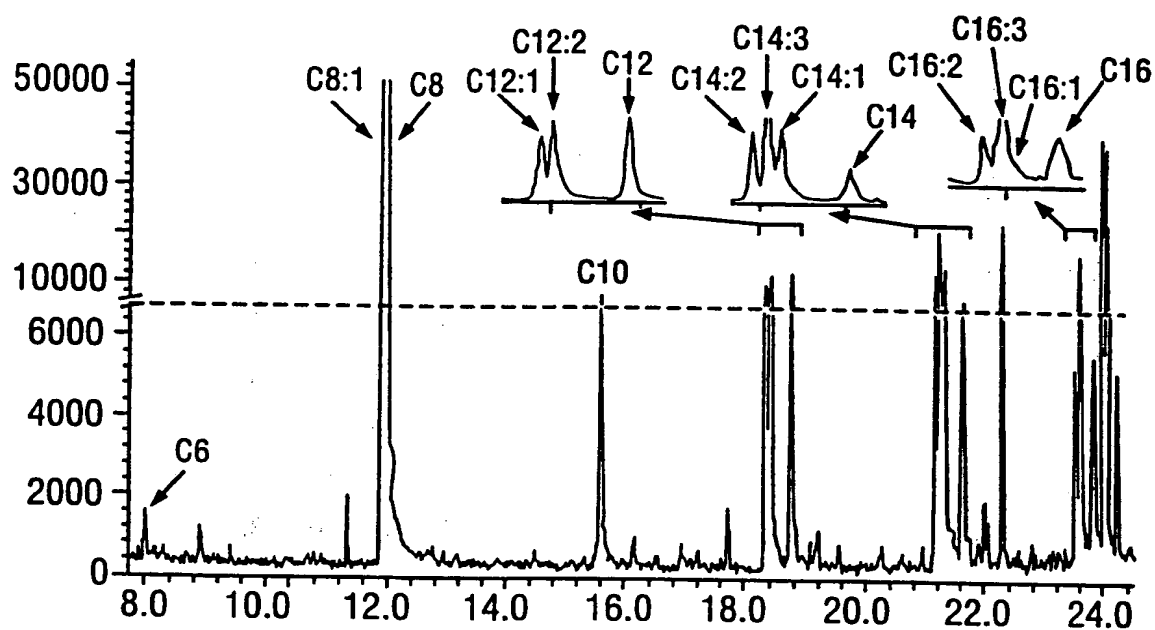


FIG. 1A

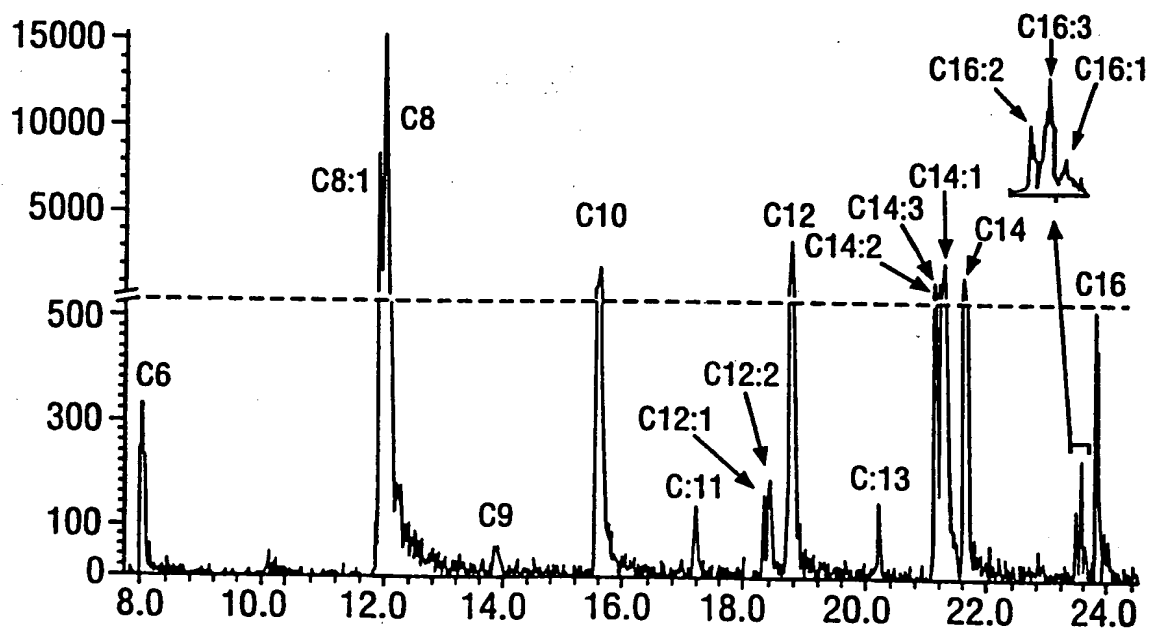


FIG. 1B



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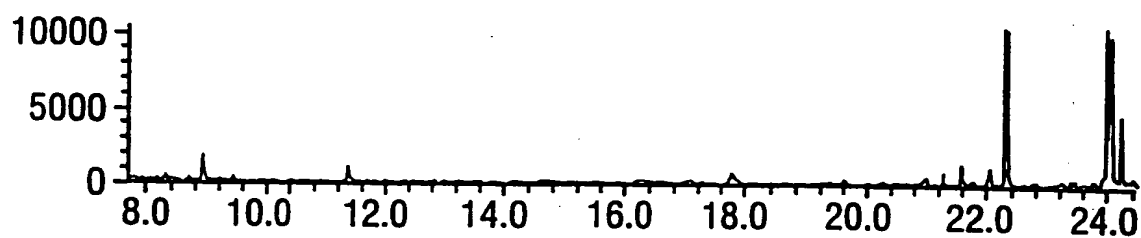


FIG. 1C

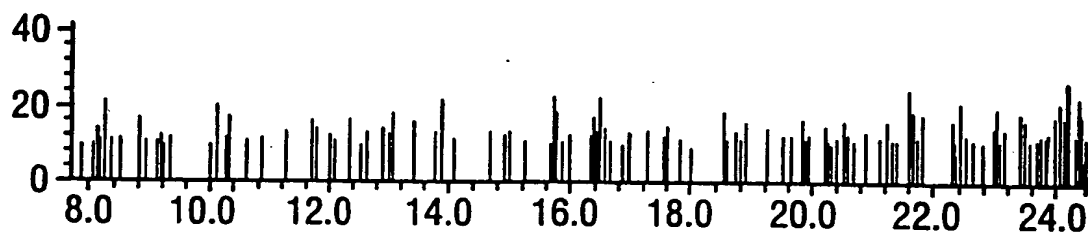


FIG. 1D

# INTERNATIONAL SEARCH REPORT

National Application No

PCT/US 98/00083

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/82 C12N15/52 C12N15/62 C12N15/81 C12P7/62  
A01H5/00 C08G63/06

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12P C12N A01H C08G

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 526 850 A (KANEGAFUCHI CHEMICAL IND) 10 February 1993 see the whole document	133
X	WO 95 33064 A (PROCTER & GAMBLE) 7 December 1995 see examples 1-4	132
X	WO 95 33065 A (PROCTER & GAMBLE) 7 December 1995 see examples 1-5	132
X	WO 97 07230 A (PROCTER & GAMBLE) 27 February 1997 see examples 1-3	132

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

### \* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
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- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

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- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "&" document member of the same patent family

Date of the actual completion of the international search

17 September 1998

Date of mailing of the international search report

29/09/1998

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# INTERNATIONAL SEARCH REPORT

International Application No  
PCT/US 98/00083

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 97 07229 A (PROCTER & GAMBLE) 27 February 1997 see example 4 ---	132
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